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Adrenocortical Insufficiency Due to Metastatic Infiltration of the Adrenal Glands

By

Johannes Hagtvet

Metastatic cancer to the adrenal glands is a common finding at necropsy. The adrenal tissue may apparently be completely replaced by tumor tissue and even on careful examination no normal tissue is found. Under such circumstances one would expect to find clinical signs of adrenal insufficiency or Addison's disease.

Thomas Addison, in his original description of 1855 interpreted 4 of 11 cases as having a carcinomatous etiology. An epecritic evaluation, however, has since proved this assumption incorrect (14).

In 1948 at Oslo Municipal Hospital, Ullevaal, the possibility of adrenal insufficiency secondary to bilateral tumor infiltration of the adrenals, was discussed (fig. 1 and 2). This was observed in a 44-year-old man who died in shock after a hernia operation. The clinical symptoms — prostration, adynamia and hypotonia — disproportionate to the surgical trauma, pointed to adrenocortical hypofunction. The final proof in the form of hormone analyses was not available at that time.

The literature previous to 1930 indicates that most investigators in this field were sceptical as to the relationship between Addison's disease and adrenal metastases. Rowntree and Snell (11) in 1931 reviewed more than 100 cases of Addison's disease and did not find a single definite case in this category. Guttman (6) in a compilation of 566 cases of Addison's disease up to 1930, found only 2 primary neoplasms and 5 metastatic. In 34 cases of bilateral adrenal metastases there were no symptoms of Addison's disease.

The usual explanation has been that the patients succumb to the primary disease or to metastases to other organs before the adrenals are completely destroyed or that the metastases are not sufficiently extensive to destroy the cortical function entirely. It has been claimed that thorough microscopic examination revealed remnants of surviving parenchyma in most cases.

Unaware of the recent literature on this subject we encountered in 1958 at Kongsberg Hospital a case in which the

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Fig 1 A 44-year-old man. Cut section. Primary adrenal carcinoma.



Fig 2. Photomicrograph f adrenal gland (fig 1)

clinical signs pointed to adrenocortical insufficiency In our opinion the treatment and electrolyte and hormone analyses confirmed this diagnosis

Case report

A 59-year-old male, who had worked as a silver miner for more than 30 years. One brother died of cancer and another of tuberculosis. Since 1918 he had been admitted to hospital 3 times for pneumonia, dyspepsia and bouts of diarrhea. In 1950 he had a brief hematuria after a motor cycle accident.

Present illness In the middle of September 1958 he became ill with fever and dedolations, and on October 26 had to go to bed. None of the usual antibiotics were effective against the fever which recurred between 39 and 40° C. He suffered from nausea and vomiting and lost 10 kg in spite of a good appetite.

On admission to the Medical Department on Nov 6 his general condition was fairly good considering his recent loss in weight. His appetite was good in spite of the persistent fever

Physical examination revealed an asthenic man who appeared pale and chronically ill. He was slightly underweight (height/weight 184 cm/75 kg) Abdominal examination revealed a palpable liver 2 fingerbreadths below the costal arch. No spleen or lymph node enlargement. There was no abnormal pigmentation. Ophthalmologic examination revealed nothing abnormal.

Laboratory examination Pirquet/Mantoux 0—0 Blood pressure 130/70 mm Hg E.S.R. 14 mm Hb 11 g/100 ml. R.B.C. 4 mill., W.B.C. 4 400—6 000/mm³ Platelets 250,000/mm³ Blood smear 1 eosinophils, 2 % stab neutrophils, 30 % segmented neutrophils, 50 % lymphocytes and 5 monocytes. The remaining 12 % were atypical cells, large monocyte like cells with blistered or vacuolized pale cytoplasm, some of them, however polynuclear Identical cells were found in the sternal marrow in addition to some smaller cells with eccentric nuclei and more darkly stained cytoplasm (fig 3)

Serum proteins 5 4 Albumin/globulin 2 7/2.7 The electrophoresis diagram showed a broad γ-stripe (fig 4) No L.E.-cells were found on repeated examination. The Wassermann, Meinicke II kl. Widal Bang, leptospirosis-Weil, Paul Bunnell reactions were negative. No organisms were cultured from the blood. Coombs test was negative.

Urine and renal functions were normal serum creatinine 1 0 mg/100 ml, non-protein-N 16 mg/100 ml. Of the hepatic function tests, the flocculation reactions, icteric index, alkaline phosphatase and cholesterol were normal. The prothrombin-proconvertin value according to Owren was 69 After injection of vitamin K it increased to 100 %. Transaminase One test gave a borderline value of SGOT 60 units/ml the others were normal.



Fig. 3. Blood smear and bone marrow film (case report). Immature myeloid cell in the peripheral blood plasma cells in the sternal marrow

Glucose tolerance. Maximum increase from 89 to 120 mg/100 ml after 1/2—1 hour followed by drop to the original value after 1 1/2— hours. Fasting values 80 mg/100 ml (Hagedorn-Jensen method).

Amylase test according to Somogyi 350—750 units. The gastric juice contained free HCl benzidine reactions in the feces were negative. Microscopically the feces contained no undigested fat or starch.

X-ray examination. Heart-lungs. Peribronchial congestion, probably bronchiectases. Silicosis. *Stomach-duodenum.* Funnel-shaped, conical formation in the sinus-canalis region with stepwise borderline toward the stomach. In central reclining position irregular rarefactions in the canalis region. Large duodenal "horseshoe"? Irregular contours on the lesser curvature in the descending part. *Tumor contracts the pancreas.*

Urography. Enlarged right kidney. Contrast filling of the lesser calices irregularly distributed and no visible pelvic ampulla. Caudal parts of the calices dislocated and the ureters quite far from the spinal column.

During his stay in hospital the patient lost weight despite good appetite and his condition changed insignificantly during the first 2 weeks. The benzidine reactions in the feces were negative. The hematologic findings remained unchanged with a low erythrocyte sedimentation rate (14 mm). The serum

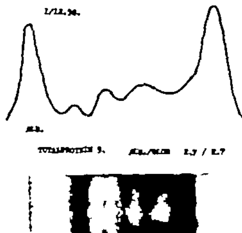


Fig. 4. Paper electrophoresis pattern of serum.

gradually became turbid and milky. No cold agglutinin or cryoglobulin could be demonstrated. Sia test negative. Antibiotics and sulfonamides in large doses, also given parenterally had no effect.

The tentative diagnosis after 2 weeks stay in hospital was a non-palpable tumor probably malignant, in the upper part of the abdomen near to the spinal column, dislocating the kidneys and the duodenal loop, and located externally to the lower part of the stomach and the pancreatic duct.



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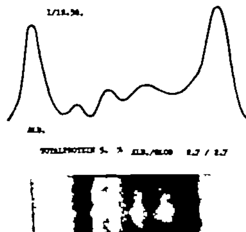


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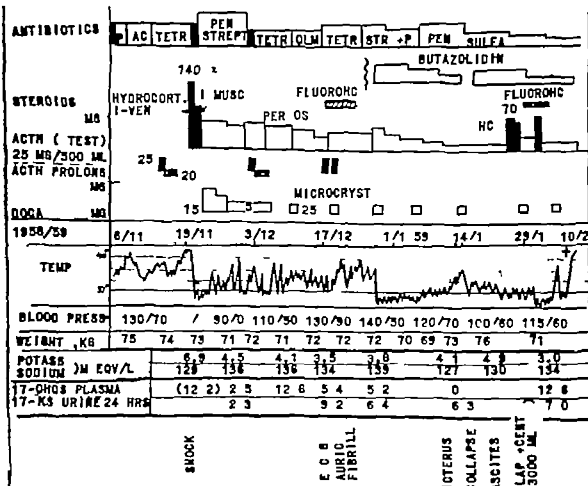


Fig 5

As his general condition deteriorated presumably because of the fever an attempt was made to take advantage of the antiphlogistic effect of the corticosteroids by administering 25 mg of ACTH in a liter of saline as an 8 hour intravenous drip, followed by intramuscular administration of ACTH for 5 days. No immediate effect was observed (fig 5)

On the 16th day after admission, 7 days after the ACTH infusion and 24 hours after a blood transfusion, the patient suddenly went into shock. He became pale, had cold sweats and was periodically mentally disturbed. Blood pressure was difficult to measure, the pulse was slow and regular (70). There was a critical drop in temperature to subnormal values (35 C)

A diagnosis of adrenocortical insufficiency was made and hydrocortisone *(140 mg twice the first day)* was administered intravenously in 2 l of saline. The shock symptoms disappeared and the blood pressure became stabilized. Injections of steroid preparations and desoxycorticosterone acetate were continued and gradually replaced by oral administration. When the shock was over the temperature began to rise again.

A blood sample taken before the ACTH treatment showed a potassium value of 6.9 mEq/l, while the sodium and chlorides in the serum were low 120 and 87 mEq/l, respectively

Tests of adrenocortical function showed the results presented in table I. The technique of Kirkeby Lingjærde (8) was employed, taking fasting samples of serum for 3 days, stimulation with ACTH by intravenous drip for 2 days and a plasma test for 17 OHCS *determination after 6 hours infusion.* The excretion of 17 ketosteroids (KS) in the urine

Table I ACTH stimulation test

	4—7/12 1958	21—23/12 1958	Normal values
Plasma (17-OHCS/6 hours, g/100 ml)			
Before ACTH	2.5	5.2	13.4—22.4
After ACTH, 25 IU i. ven., 1th day	—	9.9	30—60
After ACTH, 25 IU i. ven., 2nd day	10.4	6.4	45—70
Urine (17-KS, mg/100 ml)			
Before ACTH	2.5	9.2	14
After ACTH, 25 IU i. ven., 1th day	—	7.7	22
After ACTH, 25 IU i. ven., 2nd day	1.7	6.5	32

The patient was receiving 60—80 mg hydrocortisone per day as tablets.
According to Kirkeby-Langjerde (16).

was examined during the same period. No aldosterone was found in the urine.

Course of disease A month after admission the benzidine reactions in the feces became constantly positive, he lost weight rapidly and 2 months after admission he began having fibrillation the icteric index increased, and he became icteric. The feces became fatty and pale the alkaline phosphatase values increased (19 Bodansky units) and the prothrombin-proconvertin values decreased (56 %) After vitamin-K injection the value again reached 100 %, and subsequently remained about 70 % without vitamin K. The hemoglobin value fell in spite of blood transfusions and his general condition deteriorated rapidly Ascites developed and 3 l of a milky fluid were tapped. Histologically the centrifugate showed cells typical of malignant growth After tapping, diffuse, hard mass was palpable in the epigastrium. Phenylbutazone (Butazolidine) had prompt effect on the temperature (fig. 5)

His nails which had been slightly arched, became drum-stick shaped within 2—3 weeks. H died in cachexia 3 months after admission about 5 months after the first symptoms.

Autopsy report (Pathological Laboratory Rikshospitalet, Prof. Leiv Kreyberg, Dr E. Mylius) No abnormal pigmentation of the skin. Adherences basally in the lungs, otherwise normal air content, normal parenchyma and color Nothing abnormal in the bronchi or the hilar nodes.

The *heart* weighed 600 g Valves, myocardium and coronaries normal. The liver was large 2,500 g The surface was smooth, the cut surface showed obliterated parenchymal configurations without fibrosis. The *gall bladder* was distended and the duct dilated, filled with thick dark bile. There were 1,500 ml of ascites fluid the consistency of cream. The visceral peritoneum was smooth. The spleen was normal. The mucous lining of the stomach was hyperemic but there were no signs of ulceration, tumor or scars.

The pancreas and kidneys (fig. 6) were embedded in a large mass of greyish-yellow hard tissue which infiltrated the fatty capsule of the kidney There were hard, nodular masses



Fig. 6. Cut section of the adrenal glands with tumor infiltration of the retroperitoneal glands,

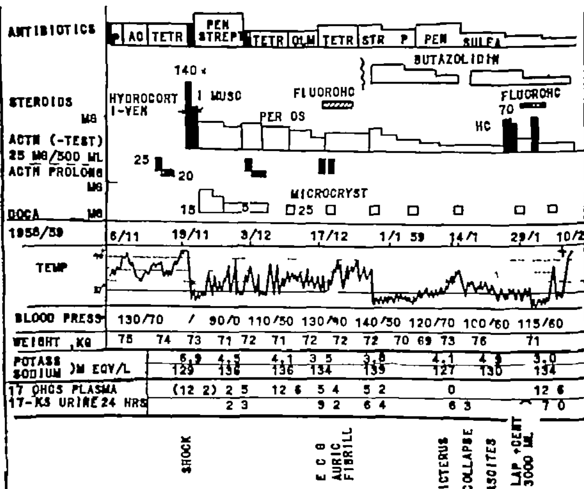


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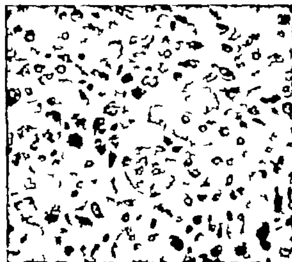
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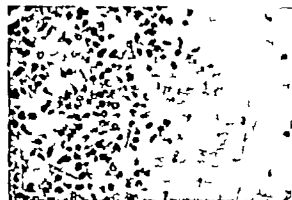
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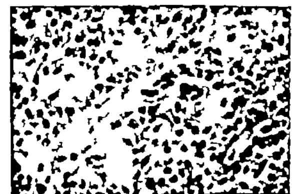
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Fig 6. Cut section of the adrenal glands with tumor infiltration of the retroperitoneal glands, pancreas and the renal fat capsule.


Fig 7 Pancreas.


Fig 8. Histological section of the adrenal gland. Low power


Fig 9 Adrenal gland High power

in the lesser omentum toward the hepatic hilus involving the bile duct. The *pancreas* was enlarged and hard with an egg-sized tumor in the head. The right kidney was enlarged. The renal pelves and parenchyma were normal.

Microscopy Pancreas In some areas both the glandular connective tissue and fatty tissue are abundantly infiltrated with small densely packed cells, separated and without any regular arrangement. The cells have sparse cytoplasm and hyperchromatic nuclei of varying form and size, but with a coarse chromatin net and many mitoses (fig 7) The tumor cells grow diffusely in the fatty tissue.

Sections from the *adrenals* show no definite remnants of connective tissue, but extensive necrosis and intense infiltration of the above described tumor tissue (fig 8 and 9) There is diffuse invasive growth in the fatty tissue. *Kidneys* intact renal parenchyma with no marked alterations. The fat capsule of the kidney however is intensely infiltrated.

The tumor tissue reveals no regular arrangement, no follicles, and there are no signs of eosinophilia. Some cells are binuclear but there are no true giant cells.

Conclusion Slightly differentiated mesenchymal tumor Infiltration in the pancreas, adrenals and the fatty capsule of the kidneys Leukemic infiltrations probable

Comments

The tentative diagnosis *intra vitam* was cancer of the pancreas with metastases to the surrounding organs. The crisis-like shock was interpreted as adrenocortical insufficiency secondary to the tumor infiltration in both adrenals with destruction of the normal tissue.

Autopsy revealed a tumor in the pancreas consisting of the same tumor tissue as that found in the renal capsule, the retroperitoneal lymph nodes and both adrenals. However the pathologists emphasize the uniform cytological picture of mesenchymal character and favour a diagnosis of leukemia.

Clinically there was no evidence of leukemia. The fever the finding of atypical cells in the blood and sternal marrow and perhaps the prompt effect of phenylbutazone (Butazolidine) (fig 5) suggested the possibility of malignant lymphogranulomatosis arising primarily from retroperitoneal lymph nodes. Even though the typical cells in Hodgkin's disease may assume deviating forms (15) the cells in this case can hardly be identified as Sternberg-Reed's cells. The autopsy afforded no reliable basis for this diagnosis either

The cytological findings together with the electrophoretic diagram were suggestive of myelomatosis (myelocytoma) The hematologic evidence, however was sparse. Sedimentation rate was normal during the entire course and there was no pathological protein in the urine.

The rapid development of gross clubbing of the fingers is also unexplained. Clubbing may however be seen in malignant diseases. The suspicion of a silent bronchial cancer or amyloidosis was not confirmed at autopsy

The pathological diagnosis is thus indefinite the origin of the tumor is unknown, but the pathologists claim that the infiltration of the adrenals is metastatic, judged by the mode of spread.

However to us the anatomical diagnosis is less interesting than the functional one the adrenocortical insufficiency after a presumed anatomic destruction of the organ. As there were neither skin pigmentation nor gastro-intestinal symptoms, it can hardly be called a case of Addison's disease, at any rate not a classical one. In a number of the more recently reported cases of Addison's disease with adrenal metastases pigmentation has also been lacking The explanation for its absence in tumor cases may possibly be the time factor — the length of time required for the insufficiency to reach full development. Guttman (6) on epicritic criteria, found that cases of Addison's disease with pigmentation as the first symptom were of longer duration than those cases in which the disease first manifested itself with adynamia or with adynamia *and* pigmentation as the dominant symptoms.

In our patient the adynamia and hypotonia before the shock were less pronounced than is usual in cases of Addison's disease of tuberculous or 'atrophic' nature. The crisis came suddenly with no premonitory symptoms with the possible exception of the fever The fever however may be due to the primary disease. It has been claimed that an ACTH infusion in a suspected case of Addison's disease may cause an 'allergic reaction with shock. ACTH stimulation should therefore be carried out during the simultaneous administration of fluorohydrocortisone. However there are different opinions on this subject. In our case the reaction came too late to be considered anaphylactoid"

A study of the adrenocortical function confirmed the diagnosis of adrenocortical failure low steroid values both before and after ACTH stimulation in urine and serum, except for the periods during which large doses of steroids, whose metabolites influence the result, were administered.

Two questions concerning the development in this case are of interest, viz. the lack of effect of ACTH administration in contrast to the striking effect of hydrocortisone upon the shock, and the reason why the patient went into crisis. The following explanation seems likely Because of the primary disease the patient was in a permanent condition of

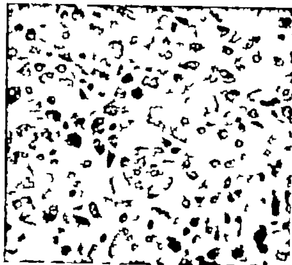


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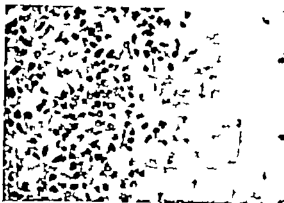


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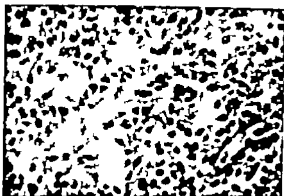


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be rare. Leary and Masters (9) found 4 such cases in the literature up to 1957 They added a fifth case. In the description of the first case it was remarkable that the adrenals presented the picture of a hemorrhagic necrosis as a result of the obliteration of the kidney vessels.

In 1958 Ribaudo (10) reported a case of Addison's disease and diabetes insipidus as a result of metastases from a bronchial cancer The author considers it the eighth case published due to adrenal metastases.

As the last decade has also brought reports of definite cases of hyperfunction of the cortex (1 2) with Cushing like electrolyte and hormone disturbances due to metastatic carcinoma, the previously accepted claim that the function of an endocrine organ is rarely if ever altered by metastases must now be considered disproved.

Guttman s 5 neoplasms in 566 cases of Addison's disease (6) are a special category as regards pathology One case was a congenital neuroblastoma in a child (Herts and Secher (7)) The tumor originated from immature sympathetic cells in the adrenal medulla with secondary destruction of the cortex. The others were lymphosarcoma originating from retroperitoneal lymph nodes (Warthin et al. (16)) lymphangioendothelioma of the peritoneum (Bannwart (3)) paraganglioma (Riemer (6)) and endothelioma (Black (6)) In the cases of Bannwart and Warthin et al, large parts of the cortex were intact, while the celiac plexus was destroyed by pressure from the tumor tissue. A similar case was reported by Bicknell (4) In addition to the cases which are usually included in most compilations, Wallach and Scharfman (14) mention several definite and doubtful cases from the literature.

Summary

A case of adrenocortical insufficiency due to replacement of the adrenal tissue by a neoplasm is reported. The tumor tissue, of mesenchymal nature also infiltrated the pancreas, retroperitoneal lymph nodes and fatty tissue.

Electrolyte and hormone analyses were similar to those found in Addison s disease during crisis. Steroid therapy relieved the shock.

If symptoms of adrenocortical insufficiency were looked for in malignant diseases, it might be found that such cases are not as rare as has been assumed previously

Adequate therapy may not prolong life, but it can alleviate much of the discomfort in the final stages.

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"stress a situation he managed to cope with only with difficulty since the amount of functioning adrenocortical tissue was significantly reduced. When exogenous ACTH was given the remaining adrenocortical tissue was unable to increase its output of corticosteroids hence the lack of effect of ACTH administration.

The ACTH given had however another effect viz. to inhibit endogenous ACTH production. When exogenous ACTH was discontinued the anterior pituitary was unable to resume its function as soon as necessary in this gravely ill patient and since no stimulus for adrenocortical secretion was available shock developed after a few hours. Later the adrenal cortex was unable to resume any function because of the complete destruction by the tumor tissue.

Our case apparently differed from Addison s disease in respect to the large doses of steroids which had to be given after the crisis in order to maintain the blood pressure and general condition The dosage was 60—100 mg of hydrocortisone daily and 25 mg of Doca every 10th day Attempts to reduce the dose caused shock like symptoms cold perspiration, pallor nausea, and hypotonia symptoms which disappeared when the dosage was increased. It was even necessary to administer parenteral steroids in order to compensate such an attempt at reducing the dosage (fig 5) The requirement for relatively large doses of steroids may be due to the patient being in a permanent state of "stress caused by the primary tumor

It is conceivable that adrenal insufficiency might be observed more frequently during the course of cancer if it were looked for at a raised suspicion threshold The symptoms are probably in most cases masked by the final stages of the primary disease. It is only when the symptoms are disproportionate to those of the primary cancer or when — as in our case — critical episodes occur that suspicion is aroused so early that the diagnosis can be verified by hormone analyses. As advanced cancer may cause both low sodium values in serum and low 17-OH and 17 ketosteroid values, the result of ACTH stimulation will be the decisive criterion for the diagnosis.

The diagnosis is not only of academic interest. Even though adequate treatment of adrenocortical insufficiency does not prolong life, it can probably alleviate the final stages for some of the patients

A review of the literature since 1950 revealed several reports on the same subject. The first definite case with biochemical evidence is reported by Butterly et al. in 1952 (5) They found 4 probable cases in the literature and added 3 definite and one probable case themselves of adrenocortical insufficiency due to adrenal metastases from bronchial cancer In the same year Wallach and Scharfman published a similar case from the same hospital (14)

In an autopsy material from the Cancer Institute of Columbia University Sahagian Edwards and Holland found 42 per cent of adrenal metastases in 383 autopsy cases of bronchial carcinoma. Of these 119 cases there were 4 with signs of adrenocortical insufficiency *intra vitam*, judged clinically and by hormone electrolyte analyses.

The frequency of adrenal metastases in relation to different cancer localizations is reported to be in the following decreasing sequence bronchial- mammary renal gastric and pancreatic cancer

Adrenocortical insufficiency due to metastases from gastric cancer is said to

be rare. Leary and Masters (9) found 4 such cases in the literature up to 1937 They added a fifth case. In the description of the first case it was remarkable that the adrenals presented the picture of a hemorrhagic necrosis as a result of the obliteration of the kidney vessels.

In 1958 Ribando (10) reported a case of Addison's disease and diabetes insipidus as a result of metastases from a bronchial cancer The author considers it the eighth case published due to adrenal metastases.

As the last decade has also brought reports of definite cases of hyperfunction of the cortex (1 2) with Cushing-like electrolyte and hormone disturbances due to metastatic carcinoma, the previously accepted claim that the function of an endocrine organ is rarely if ever altered by metastases must now be considered disproved.

Guttman's 5 neoplasms in 566 cases of Addison's disease (6) are a special category as regards pathology One case was a congenital neuroblastoma in a child (Hertz and Secher (7)) The tumor originated from immature sympathetic cells in the adrenal medulla with secondary destruction of the cortex. The others were lymphosarcoma originating from retroperitoneal lymph nodes (Warthin et al. (16)) lymphangioendothelioma of the peritoneum (Bannwart (3)) paraganglioma (Riemer (6)) and endothelioma (Black (6)) In the cases of Bannwart and Warthin et al. large parts of the cortex were intact, while the celiac plexus was destroyed by pressure from the tumor tissue. A similar case was reported by Bicknell (4) In addition to the cases which are usually included in most compilations, Wallach and Scharfman (14) mention several definite and doubtful cases from the literature.

Summary

A case of adrenocortical insufficiency due to replacement of the adrenal tissue by a neoplasm is reported The tumor tissue, of mesenchymal nature also infiltrated the pancreas, retroperitoneal lymph nodes and fatty tissue.

Electrolyte and hormone analyses were similar to those found in Addison's disease during crisis. Steroid therapy relieved the shock.

If symptoms of adrenocortical insufficiency were looked for in malignant diseases, it might be found that such cases are not as rare as has been assumed previously

Adequate therapy may not prolong life, but it can alleviate much of the discomfort in the final stages.

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Infectious Renal Disease in a Department of General Medicine

A Survey of Case Notes from 11 Years

By

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Apart from cases of typical glomerulonephritis, typical acute nephrosis, and malformation, the precise diagnosis of medical renal disease is extremely difficult. Clinically infectious renal disease is divided into two groups acute nephritis and chronic nephritis without, however it being possible to say with certainty that a chronic pyelonephritis has developed from an acute very often no history of an acute phase can be obtained from patients suffering from chronic pyelonephritis (7 8) And when Bell (2) for example, chooses to define a pyelonephritis as chronic when the symptoms have lasted for more than four months, it can be understood that the differentiation between the acute and chronic forms of the disease rests upon a very uncertain foundation. The great divergences in the frequency with which the diagnosis of pyelonephritis occurs in large autopsy materials show that the various investigators must have used different criteria for the diagnosis. Kimmelstiel et al. found that patients with chronic pyelonephritis comprised only 2.8 per cent of a material of 3,393 autopsies, whereas other authors have found this disease in 20 per cent (1), 15 per cent (11) 11 6 per cent (6) and 9 per cent (7)

In a paper published in 1961 Kimmelstiel and his colleagues write Pyelonephritis may be defined as an infectious disease of the kidney characterized by a direct inflammatory reaction of the pelvis and parenchymal interstices to the invading organisms, with secondary effects on the tubular vascular and glomerular apparatus" This definition indicates how diverse the pictures presented by pyelonephritis may be. The results of an infection of the urinary tract depend on numerous factors the nature of the infecting organism, its virulence, resistance, quantity and point of attack, the vascular state of the renal parenchyma, the state of the urinary flow and the possibility of an allergic reaction on the part of the host (8) moreover it is difficult to ascertain whether an infection is acute. Accordingly we have not attempted to divide

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Table I

Bacteria	No. of patients	♀	♂
E. coli alone	95 (60.9 %)	87	8
E. coli + other (one or more of the bacteria below)	13 (8.3 %)	6	7
G-neg non-lact.-ferm. rod	12 (7.7 %)	8	4
S albus	9 (5.8 %)	2	7
S. aureus	4 (2.5 %)	2	2
Pr vulgaris	6 (3.8 %)	6	0
Klebsiella	4 (2.5 %)	2	2
S faecalis	5 (3.2 %)	5	0
Coli- Klebsiella	3 (1.9 %)	2	1
Ps. aeruginosa	1 (0.6 %)	0	1
Mixed infection without E. coli	4 (2.5 %)	3	1
Total	156	123	33

the patients in our material into the two conventional groups of acute and chronic pyelonephritis. We have on the contrary classified our patients according to whether 1) only Escherichia coli, 2) bacteria other than E. coli or 3) other bacteria *in addition to* E coli. must be considered to have been the infecting micro-organism at the time when the investigation was carried out. We have then attempted to give an account of the clinical picture by collating for each of the three bacterial groups, the patients temperature, haemoglobin percentage, erythrocyte sedimentation rate, pathological findings in the urine renal function and length of illness, so that we have finally been able to compare the clinical pictures which had presumably been caused by each of the three groups of bacteria.

Material

In a previous paper (12) we reported that, on reviewing the case notes from this department for the years 1950—1961 we had found 1 171 case notes in which renal disease was either the main or a subsidiary diagnosis. The present paper is based upon the same material but in it we have included only those patients in whom bacteria had been cultured from a catheter or midstream specimen of urine, and in whom this urinary tract infection was the diagnosis which led to the admission of the patient. Patients with surgical diseases of the urinary tract (hypertrophy of the prostate, stricture, and nephrolithiasis) have been excluded from the material, as have those with pregnancy diabetes mellitus, and incontinence of urine. As a result of this selection the material came to comprise 156 patients, of whom 123 were women, and only 33 were men.

Of these 156 patients there were none who, as far as could be ascertained from the case notes, had been treated with antibiotics during the 14 days immediately preceding admission.

Nearly all the bacteriological investigations were carried out at Statens Serum Institut

In order to relate the bacterial findings and the clinical information as nearly as possible to each other in point of time, it is those clinical data from the period at which the urine was taken for culture that have been selected from the case histories and used in the following diagrams. As all the patients had renal disease as their main diagnosis, the various data in combination give a clinical picture of the condition of the patients on admission, before the commencement of active therapy It has not been possible, on the basis of the available case notes, to give a similar picture of the condition of the patients at discharge, as the investigations which were carried out on admission were often not repeated immediately before discharge.

Classification

In the present "uncomplicated material it can be seen (table I) that pure E. coli infections occurred in 60 9 % of the patients, and that 92 % of such infections occurred in women. Although the number of patients who suffered from infections by organisms other than E. coli is small, there is an impression that S. albus and a Gram-negative, non-lactose-fermenting rod are quite frequently the cause of uncomplicated urinary infection in the male (5) As there were only 48 patients who were *not* infected by E. coli, and as not less than eight different bacteria were responsible for the infections in these patients, each of

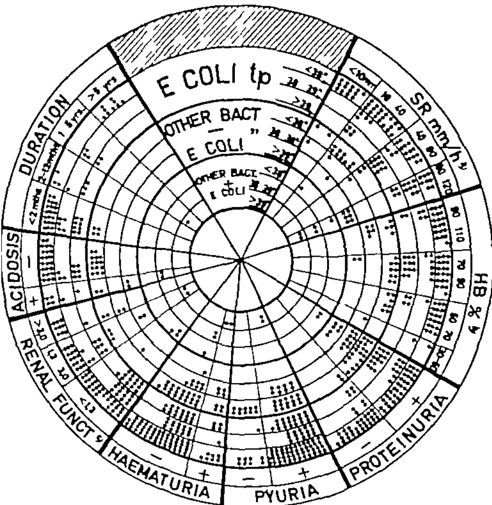


Fig. 1 Collation of the results of ESR and Hb estimations, occurrence of proteinuria, pyuria, and haematuria, result of renal function investigations, and the duration of illness in the patients, who are classified according to the bacterial findings in the urine into three groups 1) E. coli, 2) other bacteria — E. coli, 3) other bacteria + E. coli, and who at the same time had temp. < 38° 38 — 39° or > 39° C.

) ESR (mm/hour)

b) percentage of Hb in the blood,

) serum creatinine (normal = 1 3 mg/100 ml).

these eight categories would comprise so few patients that the 48 cases can hardly be divided into eight separate groups, each of which could be related individually to the clinical data. W hav therefore, as mentioned above, divided the entire material into three main groups 1) patients with pure E. coli infection, 2) patients whose urine was infected by organisms other than E. coli, and 3) patients who had other organisms in addition to E. coli in their urine.

As it is not possible to present the various relationships in a limited number of the usual coordinate systems, we have chosen to

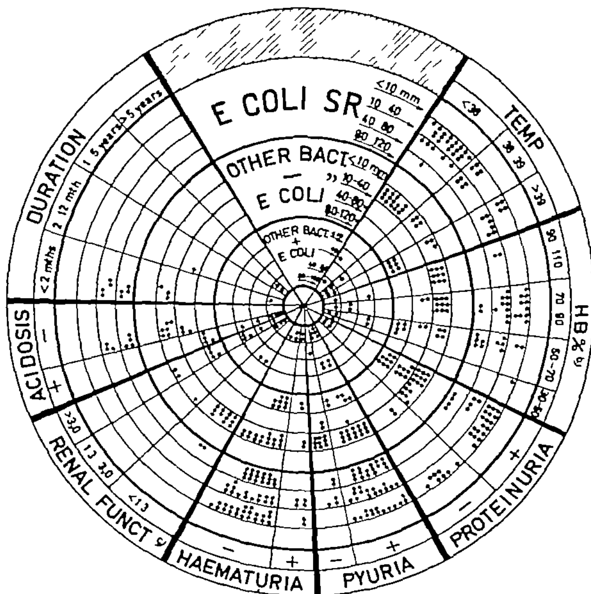


Fig 2. Collation of the temperature, Hb level, occurrence of proteinuria, pyuria, and haematuria, result of renal function investigations, and duration of illness in the patients, who are classified according to the bacterial findings in the urine into three groups 1) E. coli, 2) other bacteria — E. coli, 3) other bacteria + E. coli and who at the same time had ESR < 10 mm, 10—40 mm, 40—80 mm, or 80—120 mm/hour

b) percentage of Hb in the blood

c) serum creatinine (normal = 1 3 mg/100 ml)

present the material in the form of four circular diagrams.

The division of the material into the three groups according to the bacterial invader (as indicated above) has been adhered to in the following four diagrams, the three bacterial groups forming the constant "strain" in all the diagrams, which have by this means been divided into three concentric circular areas (see, for example, fig 1) By means of radial lines the circular areas have been divided into sectors, each of which represents a different clinical characteristic (sedimentation rate, haemoglobin percentage, etc.)

In the diagram in fig 1 the *temperature* of the individual patient (< 38° C (< 100 4 F)

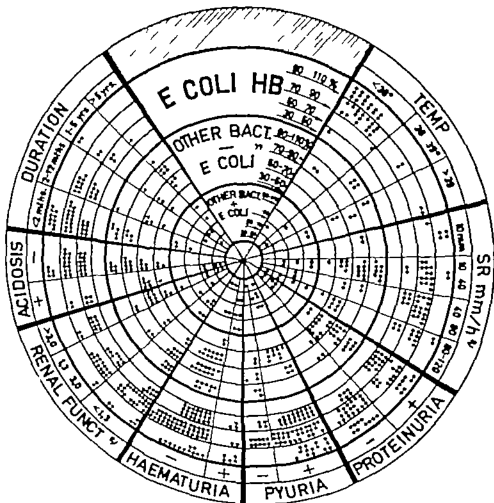


Fig. 1. Collation of the temperature, ESR, occurrence of proteinuria, pyuria, and haematuria, result of renal function investigations, and duration of illness in the patients, who are classified according to the bacterial findings in the urine into three groups 1) E. coli, 2) other bacteria — E. coli, 3) other bacteria + E. coli, and who at the same time had Hb levels of 90—110 %, 70—90 %, 50—70 %, or 30—50 %

) ESR (mm/hour);

) serum creatinine (normal = 1.3 mg/100 ml)

38°—39° C (100.4—102.2° F) > 39° C (> 102.2° F)) has been related to the various clinical values which were found in the patient concerned. In fig. 2 it is the *erythrocyte sedimentation rate* (< 10 mm, 10—40 mm, 40—80 mm, 80—120 mm/hour (Westergren)) in fig. 3 the *haemoglobin percentage* (90—110, 70—90, 50—70, 30—50 %), and in fig. 4 the *renal function* (normal, reduced, poor) that have been related to the contemporary clinical information.

Within each large sector with a heading (ESR, Hb %, etc.) each black dot represents one of the 156 patients who comprised the material, and the total number of dots within, for example, the sector headed ESR in fig. 1

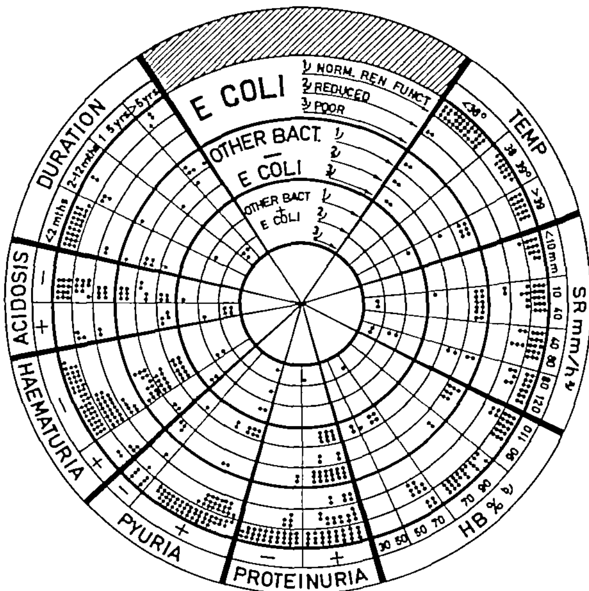


Fig. 4. Collation of the temperature, ESR and Hb levels, occurrence of proteinuria, pyuria, haematuria, and acidosis, and duration of illness in the patients, who are classified according to the bacterial findings in the urine into three groups: 1) E. coli, 2) other bacteria — E. coli, 3) other bacteria + E. coli, and who at the same time had normal, reduced, or poor renal function.

a) ESR (mm/hour)

b) percentage of Hb in the blood

1. Normal renal function: S-creatinine = < 1.3 mg/100 ml. S-urea = < 50 mg/100 ml
2. Reduced renal function: S-creatinine = 1.3—3 mg/100 ml. S-urea = 50—150 mg/100 ml
3. Poor renal function: S-creatinine = > 3 mg/100 ml. S-urea = > 150 mg/100 ml

therefore corresponds to the total number of patients (156) who had a temperature within one of the three areas < 38° C, 38°—39° C, and > 39 C, and at the same time a ESR within one of the areas < 10 mm, 10—40 mm, 40—80 mm, 80—120 mm.

Temperature

The basis for the interpretation of the diagram (fig. 1) is the three bacterial groups 1) E. coli, 2) other — E. coli, 3) other + E. coli, and the temperature 1) < 38° 2) 38°—39° 3) > 39° C. Each temperature level

follows a circular path through the various sectors.

On considering the sector representing the *ESR values*, it can be seen that the density of the dots is greatest near the periphery in all three bacterial groups, corresponding to the fact that the majority of patients had temperatures < 38° C. On consideration of the relationship between the various levels of *temperature and ESR* it becomes apparent that 17 patients had temperatures < 38° and a normal ESR. The remainder of the patients (139) had a raised ESR, 12 having ESR of 80—120 mm, despite the fact that their temperatur was < 38° When the temp. was higher than 38° there was substantial increase in the number of patients with ESR over 40 mm. It can, however be seen (in two of the present cases) that it is possible for the ESR to be normal in febrile patients.

On comparison of the levels of *temperature and Hb* it can be seen that associated with a temp. of < 38° there can be completely normal Hb values, and also all degrees of anaemia, and that this is true in all three bacterial groups. When there is pyrexia there is, particularly in the group of "pure E. coli" infections, a considerable decrease in the number of patients with normal Hb %, by far the majority of patients now lying in the region 70—90 Hb, whilst in the group other — E. coli" there is, despite the raised temperature, still comparatively large number of patients with normal Hb%.

On consideration of the relationship between *temperature and proteinuria* it is found that in the E. coli group there are equal numbers of patients without and with proteinuria in whom the temp. is < 38° whilst in the other two groups, despite a temp. < 38 there is an obvious excess of patients with proteinuria. However when the temp. is > 38 there is also an obvious excess of cases of proteinuria in the group of "pure E. coli"

A review of the relation between *temperature and pyuria* (> 5 leucocytes per high-power field) shows that the great majority of patients in all three bacterial groups had pyuria in association with temp. of < 38° In the presence of pyrexia the excess of patients having pyuria becomes even more obvious.

On comparing the temp. level with the extremely infrequent finding of *haematuria* (> 3 erythrocytes per high-power field) it can be seen that the tendency to haematuria, which is relatively more common in the groups other — E. coli" and "other + E. coli does not increase with increasing temperature.

As a number of the available case notes were from the period before the introduction of the estimation of serum creatinine the renal function has in some cases been estimated on the basis of the determination of the serum urea concentration. If an attempt is made to compare the *temperature level* with the *renal function* it can be seen that majority of apyrexial patients in the group of "pure E. coli" had normal renal function, whilst a relatively larger proportion of the apyrexial patients in the other two groups had either reduced or poor renal function. The fact that the patient was febrile *does not* seem, in the groups of "pure E. coli" and other — E. coli" to cause a reduction in renal function. The number of patients in the group other + E. coli" is so small that it is not possibl to make any comment on the apparent tendency towards reduced renal function amongst the febrile patients in this group.

As determination of the alkali reserve was carried out in only 72 patients, the total number of dots in the *acidosis sector*" is only 72 (acidosis = serum CO < 20 mEq/l).—It can be seen that only 17 patients had acidosis and that of these 9 were afebrile. There were relatively more acidotic patients in the groups "other — E. coli" and "other + E. coli" than in the group of pure E. coli

On consideration of the sector which represents the *duration of illness* (the interval between the first symptom referable to the kidneys and admission, which was known in only 138 patients) it can be seen that the apyrexial cases are evenly distributed among all four categories of duration of illness, and that the pyrexial cases show no accumulation within any particular category of duration.

Erythrocyte sedimentation rate

The basis for the interpretation of fig. 2 is the three bacterial groups and the ESR values, which are divided into ESR 1) < 10 mm, 2) 10—40 mm, 3) 40—80 mm, and 4) 80—120 mm. Although the relation between temp. and ESR has been reviewed in connection with fig. 1 the temp. values are included in this diagram for the sake of completeness.

There are very few dots in the outer areas of any of the three bacterial groups, which implies that very few patients had normal ESR.

If the sector which contains the *Hb values* is considered it can be seen that only 11 of the *total* of 19 patients with normal ESR also had normal Hb%. No less than 10 of these 11 patients belonged to the "pure E. coli" group. It was very rare for anaemic patients in any of the three groups to have a normal ESR (8 out of 111): most frequently the ESR was > 40 mm. Of the 37 patients who had ESR between 80 and 120 mm, only 6 had normal Hb.

The next sector in fig. 2 represents cases without and with *proteinuria*. Of the *total* of 19 patients with normal ESR, 9 had proteinuria. Even at higher ESR levels there are in the "pure E. coli" group almost equal numbers of patients without and with proteinuria: not until levels of ESR 80—120 mm are reached is there a definite excess of patients with proteinuria. In the group "other — E. coli" nearly all patients had proteinuria as soon as the ESR exceeded 10 mm. In the group "other + E. coli" there is also a greater tendency to proteinuria than in the group of "pure E. coli".

Consideration of the entire sector representing the cases without and with *pyuria* reveals that the great majority of patients had pyuria (122 out of 156). Only 15 of the patients with pyuria also had a normal ESR. The majority of patients with pyuria had ESR 10—40 mm. In only 5 cases (three in the E. coli group and two in the group "other — E. coli") was the ESR 80—120 mm in the absence of pyuria.

On considering the whole sector representing the cases without and with *haematuria*, it is obvious that the occurrence of haematuria is completely independent of the level of ESR.

From the sector representing *renal function* it can be seen that the ESR was normal in a *total* of 13 patients with normal renal function, 5 patients with reduced function, and only one with poor function. In all the remaining 137 cases the increase in ESR is very variable. Patients with poor renal function did not have especially high ESR.

In the sector representing the cases without and with *acidosis* it is apparent that all the acidotic patients also had a raised ESR: in the majority of cases the ESR was considerably increased.

If an attempt is made to relate the ESR to the *duration of illness* it can be seen that, at least amongst the patients in the E. coli group, a normal ESR can occur with equal frequency in patients with histories of > 5 years and in those with histories of < 2 months. Moreover it can be seen that high ESR values were not especially frequent in patients whose illness was of long duration.

Haemoglobin percentage

The basis for the interpretation of fig. 3 is the three bacterial groups and the four Hb levels: 1) 90—110, 2) 70—90, 3) 50—70 and 4) 30—50.

The distribution of the dots over the whole diagram shows that the majority of patients in all three bacterial groups had Hb percentages of either 70—90 or 50—70, as the density of dots is greatest in the areas corresponding to these values.

The relationships between Hb level, temp., and ESR have already been discussed in connection with figs. 1 and 2, so that the discussion of fig. 3 begins with the sector which represents the patients without and with *proteinuria*. In the group "pure E. coli" there are approximately equal numbers of patients without and with proteinuria, irrespective of the Hb level, whilst in the groups "other — E. coli" and "other + E. coli" there is an obvious excess of patients with proteinuria.

In the sector which shows the distribution of patients without and with *pyuria* it can be seen that the great majority of patients in all three bacterial groups had pyuria. Moreover it is common to all three groups that with decreasing Hb level there are increasing numbers of patients with pyuria.

Haematuria does not occur especially frequently in anaemic patients. Of the 28 patients with haematuria, 11 had normal Hb.

The distribution of patients in the sector representing *renal function* shows that the patients with normal Hb% had as a rule normal renal function, and that the number of patients with reduced renal function increased with falling Hb level. There are, however, exceptions, for in the three bacterial groups there are five, four and two patients, respectively, who had either reduced or poor renal

function in the presence of a normal Hb%. In addition, two patients in the "pure E. coli" group, and one patient in the group "other — E. coli" had Hb% of 30—50, despite their normal renal function.

The majority of patients with *acidosis* had considerable anaemia (Hb 50—70 %)

On inspecting the sector representing the *duration of illness* it can be seen that the majority of patients with normal Hb% are to be found in the groups < 2 months and > 5 years. Patients with Hb 70—90 % and 50—70 % are distributed more or less evenly amongst the various categories of duration of illness.

Renal function

The basis for the interpretation of fig. 4 is the three bacterial groups and the three categories of renal function 1) normal, 2) reduced, and 3) poor

The distribution of the dots in the concentric areas shows that the majority of patients in all three bacterial groups had normal renal function, the density of the dots being greatest at the periphery Moreover it can be seen that there are relatively more patients with reduced or poor renal function in the groups "other — E. coli and other + E. coli" than in the "pure E. coli" group.

As the relationships between renal function and temp., ESR, and Hb% have been discussed in connection with the previous diagrams, the discussion of fig. 4 begins with the sector *proteinuria* When the renal function is normal there are, in the group pure E. coli" rather more patients *without* proteinuria than with this sign, whilst in the group other — E. coli there is distinct excess of patients with proteinuria, even when the renal function is normal. When the renal function is reduced or poor there is the same obvious excess of patients with proteinuria in all three bacterial groups.

From the sector which represents cases without and with *pyuria* it is apparent that pyuria is such common sign that it is easy to count those patients in whom it was absent, even in the presence of normal renal function. In the group other — E. coli there are relatively more patients without pyuria than in the other two bacterial groups.

I all three groups *haematuria* is sign which tends to decrease rather than increase with decreasing renal function.

As *acidosis* is a sign of poor renal function, this sign is obviously only present when the renal function is reduced or poor However there were a number of patients who, despite the presence of reduced or poor renal function, were not acidotic.

Normal renal function is to be found most frequently in patients with *duration of illness* of < 2 months, but there are, t least in the groups of "pure E. coli" and "other — E. coli" a number of patients who had retained normal renal function despite the fact that from 2 months to 5 years had passed since the appearance of the first symptoms. Cases with reduced or poor renal function were evenly distributed among all four categories of duration of illness.

Discussion

We have no proof of how often it was in fact those bacteria which were demonstrated in the urine that were responsible for the infectious aspect of the patient's renal disease. However as the urine was in all cases a catheter or midstream specimen, and as there were at the same time massive signs and symptoms of infection, it is unlikely that the demonstrated bacteria were contaminants. At least the frequency with which E. coli occurred as the solitary bacterial finding in the urine is in close agreement with the statements of other investigators (2, 9 13 14 16) It is not however possible to say that those bacteria which were demonstrated were the *original* cause of the infectious renal disease. As many of the patients had had several recurrences, and many had therefore received previous treatment with chemotherapeutic agents, it is probable that the recurrences were produced by different strains of the same bacteria (4) Those organisms which we have called other — E. coli" comprised so many different bacteria, and occurred in so few patients, that it is uncertain which type or types of bacteria in this

group are responsible for the fact that the clinical course differs from that of an infection caused by E coli alone

One possible explanation of the more serious prognosis in infection by bacteria from the groups "other — E coli and "other + E. coli compared with infection by E coli alone is the fact that previous urinary tract infections have been treated at home with sulphonamides, without a culture having been taken from the urine. If in these cases there have been sulpha resistant organisms in the urine these have been able to multiply unhindered

The assessment of the duration of illness used here has been based upon the *information obtained from the patient* regarding the date on which he first noticed symptoms of renal disease. We have in this connection ignored the possibility that since then there may have been symptom free intervals of up to several years or that the disease, because of its progressive nature, may have been present for a longer period than that of which the patient can inform us. Due to the frequently encountered difficulty in answering this question about duration of illness, demarcations between the four subgroups (< 2 months, 2—12 months, 1—5 years > 5 years) into which the duration of illness has been divided are not naturally present and have been arbitrarily chosen Although it may occur that patients with advanced renal disease have only a short history it is, however apparent from our material that patients with reduced or poor renal function comprise a larger proportion of those patients whose renal symptoms have been present for more than 2 months than they do of the group whose symptoms have been present for less than that time.

Braude et al. (3) in experiments with rats, have observed that in some cases pyelonephritic changes become manifest only after the bacterial infection itself has disappeared This post infective tissue reaction can perhaps explain why some patients develop progressive renal disease despite the fact that the infection has apparently been completely overcome. Thus a sterile urine on discharge is no guarantee of cure (3 9 13 15 17) but the great majority of cases of acute renal infection do not give rise to recurrent or progressive renal disease (10 17)

Conclusion

The great majority of patients in all three bacterial groups had temp < 38 C (< 100 4 F) ESR < 40 mm Hb 70—90 % pyuria normal renal function, and a duration of symptoms of less than one year whilst the frequency of proteinuria varied somewhat from one bacterial group to another and haematuria was a very rare finding In all three bacterial groups there were always a few cases which deviated from the main features outlined above.

Investigation of the relationship between the various clinical data has shown that

With increasing temperature there is an increasing number of patients with high ESR, anaemia, proteinuria and pyuria, whilst haematuria, renal function, and duration of illness seem to be quite independent of the height of the temperature.

With increasing ESR there is an increasing number of patients with raised temp anaemia and proteinuria whilst pyuria occurs in the great majority of patients whatever the ESR. The occurrence of haematuria varied independently of the ESR, and similarly no relationship

has been found between ESR and renal function, or between ESR and duration of illness.

With falling Hb% there is an increasing number of patients with raised temp., raised ESR, proteinuria, pyuria and reduced renal function, whilst the occurrence of haematuria and the duration of illness bear no definite relationship to the Hb level.

With decreasing renal function there is an increasing number of patients with proteinuria and anaemia, whilst no relationship has been found between reduced or poor renal function and temp. ESR, pyuria, haematuria or the duration of illness.

In general it can be said that the patients in the three bacterial groups did not differ greatly from one another. In a few respects there was some divergency.

In the group "other — E. coli" there is relatively large number of patients who have normal Hb% despite a raised temperature.

In the "pure E. coli" group there are relatively fewer patients with proteinuria, haematuria, acidosis, and reduced renal function than in the other two groups.

Summary

From a case material comprising 1 171 case histories of patients with renal disease admitted to Department VII Municipal hospital, Copenhagen during the years 1950—1961 156 cases have been selected, being those of patients admitted with "uncomplicated" infectious renal disease, and fulfilling the criterion that the urinary tract infection should be the only diagnosis of importance on admission.

Due to the difficulties involved in the establishment of a precise definition of acute and chronic pyelonephritis, the patients have been grouped according to the bacterial findings in the urine. In the specimens of urine from these 156 patients with uncomplicated urinary tract infection the following bacteria were demonstrated: E. coli alone in 60.9 per cent, E. coli + other bacteria in 8.3 per cent, Gram-negative non-lactose fermenting rods in 7.7 per cent, S. albus in 5.8 per cent, S. aureus in 2.5 per cent, Pr. vulgaris in 3.8 per cent, Klebsiella in 2.5 per cent, S. faecalis in 2.5 per cent, Coli-Klebsiella in 1.9 per cent, Ps. aeruginosa in 0.6 per cent and mixed infection without E. coli in 2.5 per cent.

As the material available is too small to allow each of these 10 categories of infection to be related individually to the clinical material the 156 patients have been divided into three groups: patients with 1) pure E. coli infection 2) infection by bacteria other than E. coli, and 3) infection by other bacteria in addition to E. coli.

Within each of these bacterial groups we have compared the individual patient's temperature, erythrocyte sedimentation rate, haemoglobin percentage, pathological contents of urine, renal function and duration of illness.

Acknowledgement

This investigation has been carried out with support from the Fund for Advancement of Science.

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group are responsible for the fact that the clinical course differs from that of an infection caused by E coli alone.

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The Peripheral Blood Flow in Intermittent Claudication

V. Plethysmographic Studies. The Significance of the Calf Blood Flow at Rest and in Response to Timed Arrest of the Circulation

By

Leif K. Hillestad

As present venous occlusion plethysmography is regarded as the best method for estimation of the peripheral blood flow (19 25). In addition to being non-traumatic it can be applied to all parts of the extremities and provides information on both the arterial and the venous circulation.

The technical difficulties which previously restricted its use have been satisfactorily overcome in recent years. The method has therefore attained an ever increasing use also in clinical studies (1 16, 20 22, 24 27 35 40).

Important accounts on (1 5 19 41) as well as critical evaluations of (17 26, 28, 29 34 39) the classic method with water-filled plethysmographs have been published. Still more information on the method is required, however for its use in clinical research.

The present report is an analysis of the data obtained by plethysmography of the human calf and a description of how these data can be used as an index of the peripheral blood flow.

Submitted for publication December 20, 1962.

In the following the significance of the calf blood flow at rest and in response to timed circulatory arrest is evaluated. A later communication will deal with the calf blood flow in response to exercise with free and with arrested circulation.

Material and methods

The normal subjects were aged from 25 to 68 years. The patients were aged from 38 to 63 years. They all suffered from intermittent claudication of the calf due to obliterative arteriosclerosis. None of them had notable trophic lesions and in none could any other disease be demonstrated. They went through a complete clinical examination including arteriography.

In a series of patients reconstructive arterial surgery was undertaken, either a thrombendarterectomy or a by-pass procedure with a Dacron graft.

Experimental room

The room temperature was kept at 30° C in order to abolish the vasoconstrictor tone of the skin vessels and thereby keeping the skin flow

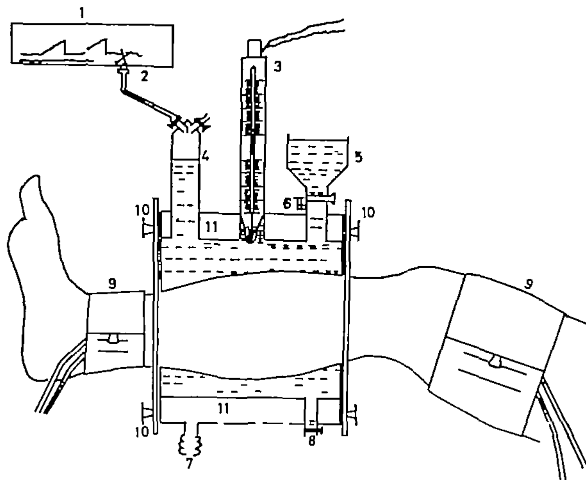


Fig. 1 The plethysmograph. 1 Multispeed kymograph 2 Piston recorder 3 Contact thermometer 4 Recording tube (chimney) with one stop-cock to the free air and the other connected to the piston recorder by a plastic tube 5 Water reservoir 6 Stop-cock for calibration 7 Inlet for hot air from a heating element regulated by the thermometer 8 Outlet for water 9 Proximal and distal cuffs 10 Wing nuts for bolting the diaphragms 11 Space for hot air surrounding the water The enclosed part of the calf is dressed by a loose sleeve of thin rubber

at a constant level. Any significant departure of the blood flow from the resting level could thus be more safely attributed to the circulation of the calf muscles. The humidity of the room was kept at 50 % (4)

Plethysmography

The principle of the method is to measure the volume increase of an organ in response to a temporary blockade of the venous outflow (9) The volume increase in the first seconds during the venous occlusion is proportional to the arterial blood flow to the organ.

The plethysmograph used in the present study was the classic water-filled steel container (fig 1) The water temperature was kept at 32 C (4) There was unlikely to be any insulation effect (37) since the high room temperature ensured a high skin temperature at the calf. Use of a stirring device was therefore considered unnecessary (18)

The sleeves (fig 1) were made of thin rubber and had diaphragms of thick rubber at both ends. The holes of the diaphragms had to fit snugly around the calf. A number of sleeves with holes of varying shapes and sizes had therefore to be available. The rubber diaphragms were bolted to each end of the plethysmograph by wing nuts and semicircular plastic plates. The holes formed by the latter had also to fit the circumference of the calf.

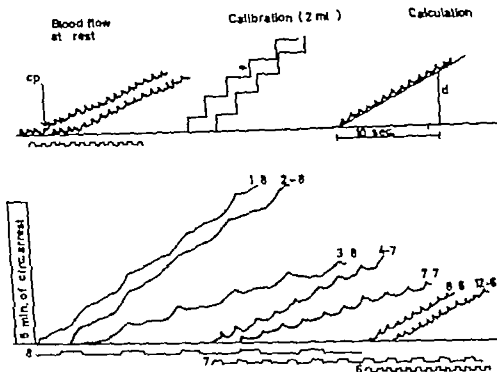


Fig. 2. The collection of volume curves. Upper half: cp indicates where the proximal cuff is inflated to the collecting pressure; is the rise in mm of the stylus on injecting 2 ml of water into the plethysmograph; d is the vertical rise of the slope in mm during ten sec. Lower half: Some flow curves collected during reactive hyperemia. The first figure of each curve refers to the time after release at which it is collected (1 is 5 sec., 2 is 20 sec. and so forth). The second figure of each curve indicates the speed of the kymograph in arbitrary units. The speed is also automatically written in sec. on the drum by means of clock-work equipped with an ink-writing stylus (below the base-line). When the slope of the curves at certain speed has fallen to an angle of 30° or less with the baseline, the speed of the kymograph is lowered one step.

A number of plastic plates were therefore needed to allow of suitable choice for the limb under examination.

A five cm wide cuff was applied distal to the apparatus and inflated to 250 mm Hg at least 15 sec. before each recording in order to exclude the foot blood flow (17, 26). The proximal cuff was 10 cm wide and enclosed in non-distensible bandage, which was bound little tighter at the distal end than at the proximal (19). Care was taken that any pressure on the skin was avoided. The cuff could be quickly inflated from pressure reservoir (19), which then could be disconnected from the cuff and adjusted to the pressure needed for the next inflation. The proximal cuff was used for the collecting as well as for the occluding pressures (33).

On application of the collecting pressure the volume increase of the calf caused the water to rise in the recording tube, and the consequent displacement of the air above was transmitted to counterbalanced spirometer (piston recorder) equipped with an inkwriting stylus. The cuff was deflated after 5—10 sec. and not inflated again until at least 10 sec. had passed (28). In the interval the free stop-cock of the recording tube was opened for a short while in order to maintain atmospheric pressure in the system. At the same time the water level in the tube was adjusted to the original level if necessary.

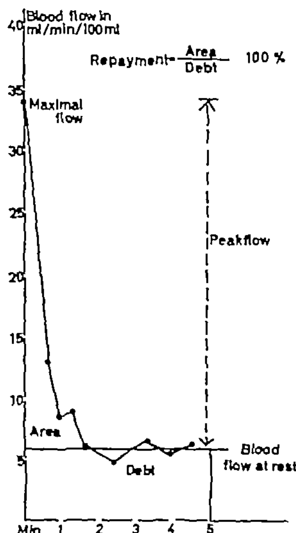


Fig. 3. The graph constructed for each experiment to calculate the excess flow following circulatory arrest. Area is commensurate with excess blood flow or reactive hyperemia flow. Area and debt are measured by means of a planimeter

Usually collecting pressures covering a wide range gave identical volume curves (19 28) and a pressure somewhat below the diastolic blood pressure was selected for the experiment (25)

In assessing the blood flow at rest the mean volume increase of several curves collected over at least 10 min. was used.

In arresting the circulation the proximal cuff was inflated to 250 mm Hg. On release of the arrest the pressure was quickly lowered to the collecting pressure and the first volume curve obtained within 5 sec. The next curve was obtained at 20 sec. and thereafter curves determined at 20-sec. intervals until 2 min. The interval was then prolonged to 30 sec. and the determination continued until the hyperemia had subsided.

The first three curves were recorded at a collecting pressure about 25—30 mm Hg lower than the original one (33 38). Two or three reactive hyperemias were registered in each patient and the mean used for the present paper.

The calibration was done during the arrest by injecting two ml of water in successive steps and measuring the mean rise of the stylus (n in fig 2). The volume of the enclosed calf was obtained by measuring the amount of water in the plethysmograph at the end of the experiment and subtracting this amount from the known volume of the apparatus. The experimental constant was derived from the formula $k = \frac{n\ 6}{2\ V/100}$ where n is the rise of the stylus in mm for each 2 ml of water. 6 is introduced to get the flow per minute as the rise of the slope is recorded for a period of 10 sec., 2 refers to the amount of water in ml used for calibration, V is the calf volume in ml, divided by 100 to obtain the flow per 100 ml of tissue. The blood flow is obtained by multiplying the rise of the slope in 10 sec. (d in fig 2) for each curve by the experimental constant. Blood flow = k. d ml/min./100 ml tissue.

The flow values were plotted into a coordinate system (fig 3) where the shape of the hyperemia could be studied and its size measured by means of a planimeter.

The subject under examination rested comfortably on a couch, the calf being in the horizontal position supported by pads of rubber foam. The level of the calf was kept in line with the sternal angle so that the veins were collapsed. The subjects were naked except for a linen cloth over the genital area and the abdomen. They remained at rest for one hour before the examination was started.

Some remarks are required on the technique of plethysmography. It is obvious that air bubbles within the apparatus may cause an uncontrolled reduction of the actual volume increase. When filled with water and before any recording the plethysmograph is tapped and moved carefully from side to side in order to make the air ascend into and out of the recording tube. For the same purpose the

calibration syringe is withdrawn several times, and any air is let out and replaced by water which is injected. To prevent formation of large air bubbles it is sometimes useful to add a little detergent to the water

Venous movement artefacts can be avoided by readjustments of the proximal cuff and by more rapid inflation of the latter to its final pressure (19 23)

The great advantage of the water-filled plethysmographs over the gas-filled ones is that the former give control of the local temperature. This is indispensable for securing stable skin flow The water-filled apparatus are moreover superior with regard to leaks, which are easily detected. However small leaks of the whole system should always be searched for The stylus should then be placed few inches above the basal position on the drum with the free stop-cock open. The latter is then shut, and the kymograph moved on for some time. A leak is observed by a downwards drift of the line on the drum.

A source of constant error is bulging of the thin rubber sleeve through the ends of the plethysmograph. An uncontrolled part of the actual volume increase may thereby be lost. In the present work thin, firm pasteboard rings were cut to fit the circumference of the calf and fastened between the rubber and the plastic diaphragms. The small space between the inner margin of the pasteboard was filled with foam rubber The effectiveness of this procedure was tested by closing the system and rapidly injecting 10 ml of water If the water in the chimney (fig 1) rose from one mark to another the distance between which corresponded to 10 ml, the plethysmograph was considered free from bulging

Table I The calf blood flow at rest. Mean values from 48 normal limbs and 89 limbs with intermittent claudication. Blood flow in ml/min/100 ml

	Normal limbs	Ischemic limbs
Blood flow at rest	3.6	3.4
S.D.	1.3	1.0
Range	1.5–6.5	1.5–7.0

Table II The calf blood flow at rest before and after successful reconstructive surgery in limbs with intermittent claudication. Blood flow in ml/min/100 ml

	Calf blood flow at rest	
	Before op.	After op.
	2.4	2.4
	3.0	5.5
	2.4	3.0
	6.0	6.5
	3.5	5.5
	2.2	3.8
	5.0	5.6
	2.7	7.2
	2.0	5.7
	3.5	3.5
	4.0	8.5
	4.2	5.4
	4.8	2.4
	2.0	2.0
	2.1	2.2
	1.0	1.5
	2.5	2.1
	2.2	3.5
	2.4	3.4
	4.4	4.5
Mean	3.2	4.4

Results

The calf blood flow at rest

When allowance is made for bone tendons and fat the calf consists of 85 % muscle and 15 % skin (2) The skin flow depends on the local and ambient temperature and increases somewhat with age (21) In comparing the absolute figures below with those of other series these two factors must be considered.

It is reasonable to assume that the capacity of muscular circulation will become apparent only at exercise or in response to stimulation of other kinds. This is indeed correct (table I, fig 10) The blood flow at rest is of similar magnitude and range in both normal and

f

Table III The spontaneous variation of the resting calf blood flow of ten limbs with constant intermittent claudication in the course of four months Blood flow in ml/min/100 ml. Variation coefficient (V c.) in % for the individual limb and for the whole group of limbs

	Months				G.	S. d.	V c.
	1	2	3	4			
	4.5	3.8	2 0	2.5	3.2	1 15	36.00
	4.0	3 0	1.8	2.7	2.8	0.91	32.50
	3.2	2.5	2.9	2.5	2.7	0.34	13.00
	2.0	2.5	3.3	3.5	2.8	0 70	25.00
	3.5	7 4	3.0	4.5	4 6	1.96	42.60
	3 0	3.2	3.0	2.8	3.0	0 17	5.70
	3.0	2.2	2.5	2.5	2.5	0.33	13.20
	2.2	3.5	2.4	2.3	2.6	0.60	23.00
	2 4	3.0	3.0	3.0	2.8	0.30	10.70
	2 4	2.0	2.2	2.4	2.2	0.20	9.10
Mean ind. var	3.0	3.3	2.6	2.9	2.9	0.67	21.1 %
Monthly var group	3.0	3.3	2.6	2.9	2.9	0.30	10.3 %

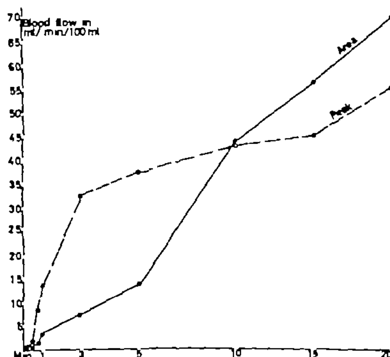


Fig 4 The reactive hyperemic flow of the calf following increasing periods of circulatory arrest in two normal subjects.

ischemic limbs. With very advanced occlusive disease the blood flow at rest may be high (case 4 in fig 8) but the blood flow at rest provides little information on the patency of the vessels.

This is supported by evaluating the changes of the resting flow following successful reconstructive surgery of ischemic limbs (table II) In several cases the resting flow remains unaltered or is even

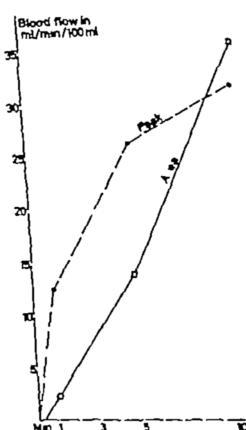


Fig. 5 The reactive hyperemic flow of the calf following increasing periods of circulatory arrest in ten normal subjects.

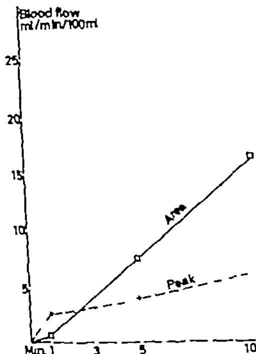


Fig. 6. The reactive hyperemic flow of the calf following increasing periods of circulatory arrest in ten patients with intermittent claudication of the calf.

decreased after the operation. The mean increase of the resting flow is 40 which is significant change (table III) However the potential increase of the circulation is about 350 % (table VI) It should also be observed that the resting flow is within the normal range both before and after the operation

In evaluating the effect of any vasoactive procedure it is useful to know the spontaneous variation of the resting flow (table III) However it must be kept in mind that any change in the resting flow does not prove any change in the capacity of the circulation.

The reactive hyperemia test

Reactive hyperemia means the increased blood flow following a period of ischemia (29 39 41) and is a response occurring in both skin and deeper tissues independently of any nervous elements.

If the normal calf is deprived of its blood supply for increasing periods of time (fig. 4) the resulting hyperemias may attain huge dimensions. The peak flow which merely reflects the caliber of the vessels, reveals the great ability of the latter to dilate. Moreover the peak flow increases rapidly at first, and later its slope flattens off. It appears, however that the maximal dilatation has not been obtained even in response to 20 min. of circulatory arrest. The total hyperemia flow (excess

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	Months				G₀	S. d.	V c.
	1	2	3	4			
	4.5	3.8	2.0	2.5	3.2	1.15	36.00
	4.0	3.0	1.8	2.7	2.8	0.91	32.50
	3.2	2.5	2.9	2.3	2.7	0.34	13.00
	2.0	2.5	3.3	3.5	2.8	0.70	25.00
	3.5	7.4	3.0	4.5	4.6	1.96	42.60
	3.0	3.2	3.0	2.8	3.0	0.17	5.70
	3.0	2.2	2.5	2.5	2.5	0.33	13.20
	2.2	3.5	2.4	2.3	2.6	0.60	23.00
	2.4	3.0	3.0	3.0	2.8	0.30	10.70
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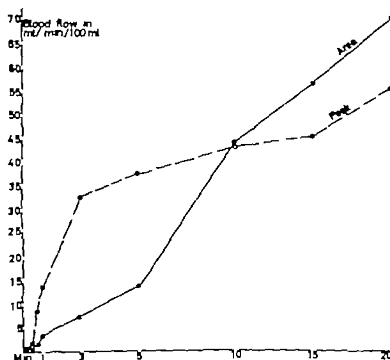


Fig. 4 The reactive hyperemic flow of the calf following increasing periods of circulatory arrest in two normal subjects.

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This is supported by evaluating the changes of the resting flow following successful reconstructive surgery of ischemic limbs (table II) In several cases the resting flow remains unaltered or is even

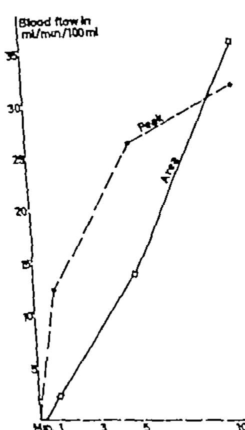


Fig. 5 The reactive hyperemic flow of the calf following increasing periods of circulatory arrest in ten normal subjects.

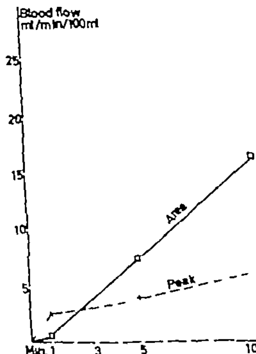


Fig. 6. The reactive hyperemic flow of the calf following increasing periods of circulatory arrest in ten patients with intermittent claudication of the calf.

decreased after the operation The mean increase of the resting flow is 40 % which is a significant change (table III) However the potential increase of the circulation is about 350 % (table VI) It should also be observed that the resting flow is within the normal range both before and after the operation.

In evaluating the effect of any vasoactive procedure it is useful to know the spontaneous variation of the resting flow (table III) However it must be kept in mind that any change in the resting flow does not prove any change in the capacity of the circulation.

The reactive hyperemia test

Reactive hyperemia means the increased blood flow following a period of ischemia (29 39 41) and is a response occurring in both skin and deeper tissues independently of any nervous elements.

If the normal calf is deprived of its blood supply for increasing periods of time (fig 4) the resulting hyperemias may attain huge dimensions. The peak flow which merely reflects the caliber of the vessels, reveals the great ability of the latter to dilate. Moreover the peak flow increases rapidly at first, and later its slope flattens off. It appears, however that the maximal dilatation has not been obtained even in response to 20 min. of circulatory arrest. The total hyperemia flow (excess

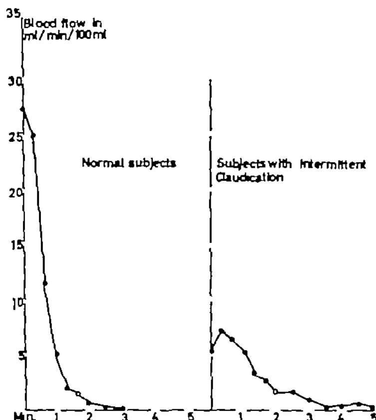


Fig. 7 The reactive hyperemic blood flow of the calf following 5 min. of circulatory arrest in five normal subjects and five patients with intermittent claudication of the calf. The repayment of the normal limbs was 9? and of the diseased limbs 53 %

flow or area) shows virtually a reciprocal relationship to the peak flow increasing slowly at the start and faster when the peak flow flattens off.

This experiment was carried out to find the time of arrest which could provoke the maximal vascular response. However in extending the arrest to 30 min untoward shock like reactions occurred in both subjects during the last period of the arrest. For this reason it was considered safe to employ only shorter periods of arrest in patients with vascular diseases.

In the further analysis of the hyperemic response to timed arrest marked differences between the normal and the diseased circulation become apparent (fig 5 and 6)

The slopes of the peak flow and the excess flow show interrelationships like those above. The excess flow largely follows a line of 45 or a little more, signifying a quantitative repayment or an overpayment of the incurred debt.

The pathologic peak flow shows a negligible increase in response to the arrests and thereby reflects the poor circulation. The excess flow follows a line of about 22 5 and a constant underpayment of the incurred debt is present throughout the experiment.

Besides these striking differences between the normal and the diseased circulation it will be observed that the crossing point between the peak flow and the excess flow is displaced to the left in the ischemic limbs. From this point on the hyperemias grow mainly by increasing the excess flow while the increase of the peak becomes relatively less. The hyperemias therefore become protracted and this change of shape occurs earlier in the diseased circulation

Yet other information is provided by these experiments. In the event that the peak flows were equal in two independent circulatory systems, the great excess flow would reveal the pathologic one. Similarly in the case of equal excess flows the pathologic one would be recognized by the relatively smaller peak flow

Furthermore it appears that a 5-min. arrest of the circulation is suitable for distinguishing between normal and ischemic limbs. Shorter arrests produce such small hyperemias in the disordered circulation that technical errors may interfere significantly Longer arrests may be harmful in patients with very advanced disease.

A 5-min. arrest gives a good differentiation (fig 7) The pathologic hyperemia is initially lower and somewhat prolonged. The delayed peak flow reflects the resistance to flow offered by the stenotic arteries and the collaterals. The pathologic excess flow is also less than normal and gives a repayment of 53 % against 96 of the normal hyperemia.

The experiments moreover demonstrate the necessity of recording the whole hyperemia with as frequent observations as possible. It is not safe to assess the hyperemic response by estimating the flow at a certain time after release (5 22, 27 40) The real differences may then easily be obscured.

The reactive hyperemia test is useful in classifying the circulation (fig 8) The advance of obliterative arterial disease is followed by a corresponding change of the hyperemia as described above. An example of excellent collateral flow is presented (curve 2 fig 8) The peak flow is within normal limits, but distinctly delayed, and the excess flow is abnormally large. In very advanced occlusive disease the peak flow can be considerably delayed (curve 4 fig 8) and the initial part of the hyperemia may be lower than the resting flow

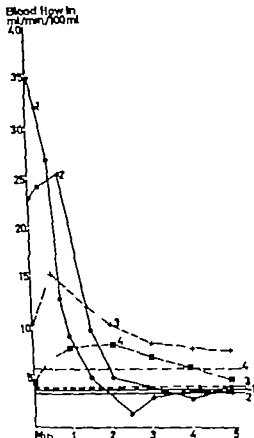


Fig. 8 The hyperemic blood flow of the calf following 5 min. of circulatory arrest t various stages of obliterative arterial disease. 1 Normal circulation 2 Complete obliteration of the femoral artery with excellent collateral flow 3 Complete obliteration of the femoral artery and marked stenosis of the popliteal artery 4 Complete obliteration of the femoral artery marked stenosis of the popliteal artery and marked involvement of the crural arteries. Note that the initial flows are lower than the resting flow and that the peak flow is considerably delayed.

The reactive hyperemia test is moreover useful in controlling treatment (fig 9) The absence of collateral flow after

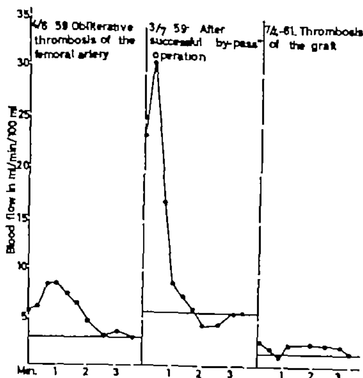


Fig 9 An example of the information obtained by the use of the reactive hyperemia test. The last examination was made four days after the sudden failure of the graft and demonstrates the absence of any collateral circulation.

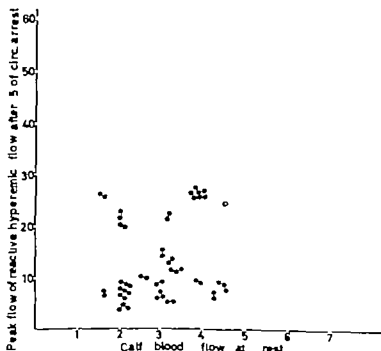


Fig 10 The calf blood flow at rest and following 5 min. of circulatory arrest (peak flow) in normal limbs (○) and in limbs with intermittent claudication (●) Blood flow in ml/min./100 ml.

acute failure of the graft is obvious as compared with the pre-operative examination. The blood flow at rest is evidently a useless index of the actual blood supply in these examples of practical plethysmography

From trial of the reactive hyperemia test in a series of normal and ischemic

Table IV. The hyperemic calf blood flow following 5 min of circulatory arrest. Mean values from 76 experiments in 48 normal limbs. The area of the hyperemic flow curve divided in three portions. Blood flow in ml/min/100 ml

	Excess blood flow (area)			
	1th min	2nd min	3rd + 4th + 5th min	Peak flow
Mean blood flow	10.8	1.8	1.3	28.2
S. D	3.6	0.5	0.4	7.6
Range	6.2–18.2	1.3–5.1	1.0–2.1	13.0–48.0

Table V. The hyperemic calf blood flow following 5 min of circulatory arrest. Mean values from 58 experiments in 54 limbs with intermittent claudication. The area of the hyperemic flow curve divided in three portions. Blood flow in ml/min/100 ml

	Excess blood flow (area)			
	1th min	2nd min	3rd + 4th + 5th min	Peak flow
Mean blood flow	6.2	2.9	2.1	9.3
S. D.	2.6	0.9	0.8	4.4
Range	1.7–14.5	0.9–3.5	0.7–4.0	3.0–22.0

Table VI. The hyperemic calf blood flow before and after successful reconstructive surgery in twenty limbs with intermittent claudication. Mean values are given in the table. Blood flow in ml/min/100 ml

	Excess blood flow			
	1th min	2nd min	3rd + 4th + 5th min	Peak flow
After op.	11.1	2.8	1.0	23.1
Before op.	4.3	2.4	1.2	6.7

limbs (fig. 10) some conclusions are warranted. A peak flow less than 15 ml/min./100 ml is definitely pathological. Around 20 ml/min./100 ml a certain overlapping between normal and pathologic values is present, but not very marked. This is probably due to the fact that patients with early and symptomless obliterative disease are not represented. The diagram indicates that these patients can be

Table VII. The repayment of the incurred debt during five minutes of circulatory arrest in the calf. Mean values from normal limbs and from ischemic limbs before and after reconstructive arterial surgery

	Blood flow at rest (ml/min/100 ml)	Excess blood flow (ml/min/100 ml)	Repayment of debt (%)
Normal limbs	3.6	13.5	103
Ischemic limbs			
Before op.	3.2	7.9	63
After op.	4.4	14.9	90

picked out by means of plethysmography. Another group of patients is also absent from the diagram, namely those with very advanced disease. Their resting flows are usually high and their peak flows very low. These flow values would have been situated in the right, lower

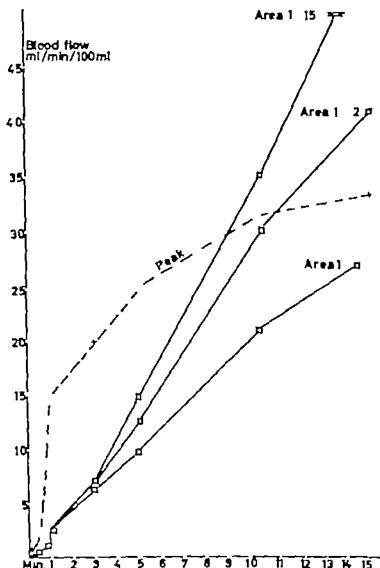


Fig 11 The peak flow and the different parts of the excess flow curve in response to increasing periods of circulatory arrest. From ten normal limbs.

corner of the diagram Finally this diagram clearly demonstrates the similarity in the resting flows of normal and ischemic limbs

In the main, the peak flow is a reliable index of the actual blood supply However its significance may become reduced by some events. Firstly the peak flow may easily be recorded too low unless the technique is perfect (1 2 33 38) Secondly the delay of the peak flow is difficult to include in the calculations. Thirdly the peak flow may be quite inadequate in certain respects, even when assisted by the total hyperemia This is shown by the following analysis, which aimed at finding a reliable way of demonstrating an improvement of the circulation.

In the normal hyperemia the main part of the flow is confined to the first minute after release (table IV) As expected the normal values have a great range due to individual differences in body build development of muscles, training blood pressure and other factors.

The pathologic hyperemia differs from the normal one mainly by a lower flow

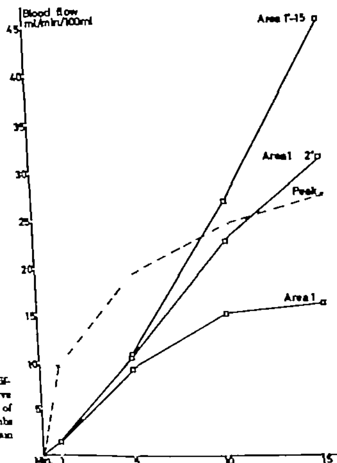


Fig. 12. The peak flow and the different parts of the excess flow curve in response to increasing periods of circulatory arrest. From six limbs with incomplete stenosis of the main artery

in the first minute (table V) The range here refers to the variable arterial involvement in the cases included.

These normal and ischemic limbs belong to two populations. It is relevant therefore to study a number of limbs prior to and after removal of the main artery obliteration (table VI) Here also the improved circulation has led to an increase mainly of the flow in the first minute. Next to the peak flow it is this stage of the hyperemia that best reflects the improvement. The ratio of the first minute flow to the rest of the hyperemia increases from 1.2 before operation to 3.1 afterwards. The corresponding ratio of the peak flows is 3.5. In the normal limbs (table IV) the ratio of the first minute flow to the remaining part of the hyperemia is about 3.5

The total hyperemia and the repayment are likewise increased after the operation (table VII) The increase of the repayment is small and does not reflect the actual improvement. This is due to the simultaneous increase of the resting flow which is caused mainly by an increased skin flow (16 35) The improve

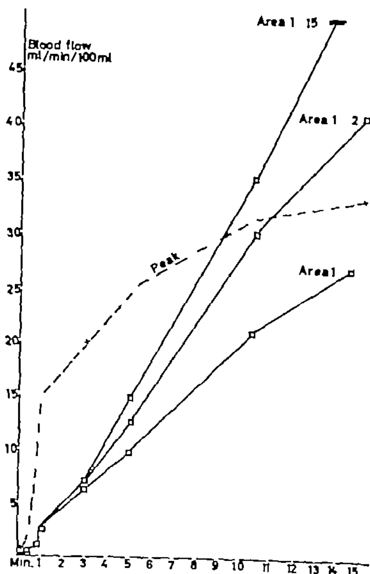


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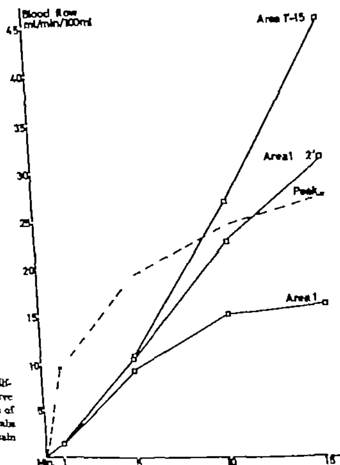


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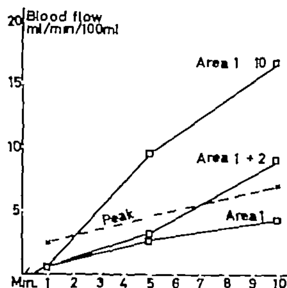


Fig 13 The peak flow and the different parts of the excess flow curve following increasing periods of circulatory arrest. From five limbs with complete stenosis of the main artery

ment is better reflected by calculating the repayment of the first minute of the hyperemias This gives a repayment in the normal and the ischemic limbs before and after operation of 400 % 112 % and 340 % respectively

In conclusion, an improvement is signified by an increase of the peak flow and an increase of the first minute flow in relation to the rest of the hyperemia.

This analysis of the composition of the hyperemia may be brought a step further by the following experiments (fig 11 12 and 13)

In response to increasing arrests the normal hyperemia increases rapidly (fig 11) with an overpayment of the incurred debt. This amounts to 107 % 130 % and 144 % following 5 10 and 15 min. of arrest. However it is the changed composition of the hyperemia which should be recognized. The first minute flow does not increase as fast as the remaining part of the hyperemia. The ratio of the first minute flow to the rest diminishes throughout the experiment. Even the normal hyperemia therefore becomes protracted

In the limbs with incomplete stenosis (fig 12) and complete stenosis (fig 13) the peak flow and the hyperemia show a much smaller increase. This has been shown earlier It is the changing shape of the hyperemia that should be studied. It will then be observed that the same alterations as described in the normal hyperemia occur but earlier and more marked the poorer the circulation is. In the limbs with incomplete stenosis the hyperemia is already protracted from the start, and the first minute flow shows only a small rise in the experiment. With regard to the hyperemia of the normal limbs and the ischemic limbs there seems to be a halfway stage between the normal and pathological circulation.

With increasing arrest the blood flow increases, but this does not represent an improvement of flow because the tube is unchanged It is a vasodilatation which is characterized by an increase of the peak flow an increase of the total hyperemic flow and by a fall of the ratio between the first minute flow and the flow during the remaining part of the hyperemia.

The results of the above investigation can now be reviewed The vasodilatation occurring in response to increasing periods of ischemia represents an increased blood flow but not an improvement of the flow Both vasodilatation and improved circulation have in common an increase of the peak flow and an increase of the total hyperemia. They can only be distinguished from each other by a quantitative assessment of the change in the composition of the hyperemia. In vasodilatation as defined here the ratio of the first minute flow of the

Table VIII. The behavior of the different parts of the hyperemia following increasing periods of circulatory arrest. Mean values from 10 normal limbs, 6 limbs with incomplete stenosis and 5 limbs with complete stenosis of the main artery. The excess blood flow (hyperemia flow) of the calf in ml/min/100 ml (ml)

Circulatory arrest	Excess blood flow of limbs with					
	Patent art.		Incompl. stenosis		Complete stenosis	
	ml	%	ml	%	ml	%
5 min						
1st min flow	10.0	70	9.4	87	2.5	26
2nd min flow	2.5	17	1.1	10	1.0	11
3rd–5th min flow	2.0	13	0.3	3	6.0	63
Total	14.5	100	10.8	100	9.5	100
10 min						
1st min flow	21.0	60	15.0	54	4.0	24
2nd min flow	9.0	26	7.7	28	5.0	29
3rd–10th min flow	5.0	14	4.0	18	8.0	48
Total	35.0	100	26.7	100	17.0	100
15 min						
1st min flow	27.0	47	16.0	36	–	–
2nd min flow	14.0	24	13.0	33	–	–
3rd–15th min flow	17.5	29	14.2	31	–	–
Total	58.5	100	35.2	100	–	–

hyperemia to the rest of it becomes less. In a true improvement of the circulation the ratio becomes greater.

A satisfactory control of a procedure aimed at improving the flow requires therefore that both the peak flow and the above stages of the hyperemia are assessed and evaluated.

The information obtained by stimulating the circulation by means of increasing periods of arrest (fig. 11, 12 and 13) may be better understood by giving the

Table IX. The spontaneous variation of the hyperemic calf flow in the course of 4 successive months. A group of ten patients with constant intermittent claudication was studied. Mean values for the variation of the individual limb hyperemia as well as for the hyperemia of the group are given in the table

	Variation coefficient (%)	
	Individual	The group
Peak flow	18.8	4.5
1st min area	19.4	7.6
2nd min area	17.5	2.6
3rd–5th min area	20.6	7.6

figures and showing the percentual relationships between the different stages of the hyperemia (table VIII). By reading the table from above downwards the flow patterns of vasodilatation become apparent. By reading from right to left the corresponding patterns of improvement become evident. An exception is to be made for the response to five minutes of ischemia in the limbs with incomplete stenosis, as the shape of the hyperemia here is not different from the normal one. Probably the stimulus is too weak to provoke any distinction. It is known that a marked constriction of an artery is required to bring about a decrease of flow (31). Longer periods of arrest provide, however, a satisfactory distinction. It seems that by employing longer arrests than five minutes a better diagnostic approach can be obtained in the early cases of obliterative disease.

A complete analysis of the reactive hyperemia test also includes a study of its spontaneous variation (table IX). The study shows that for a small group of patients the test provides so stable results that it is suitable even for long-term investigations.

Comments

In a large series the present work confirms earlier reports (1 13 16 35) in demonstrating that the blood flow at rest in the skeletal muscle is independent of the capacity of the circulation

This is in accordance with the finding of an autoregulation of the resting blood flow in skeletal muscles of the animal (36) The resistance of the muscle vessels varies in proportion to the perfusion pressure and the flow so that the resting flow remains constant over a wide range of pressures. Thus the reduced pressure in the ischemic limb (13) is probably the factor initiating the autoregulatory mechanism

The relatively high flow at rest observed in limbs with very advanced occlusive disease is of obscure origin. It may merely be a result of an increased skin flow only like that described in hands with similar disorders (24) Metabolites and oxygen lack may also be responsible (10 43) by enforcing the vasodilatation beyond that produced by the local pressure fall. This is supported by the finding that in such limbs the arterio-venous oxygen difference is increased (30) while this is not the case in limbs with less advanced occlusive disease.

After all the significant fact is that the blood flow at rest of the human calf is almost unaffected by the patency of the main arteries. A change of the resting blood flow is therefore by no means linked with any corresponding change of the potential blood supply

In contrast to the resting flow the hyperemia following timed circulatory arrest (3 7 29) is an excellent index of the capacity of the circulation The present report has probably in this respect added some information to that already existing The findings need a short discussion in view of the current concept of the reactive hyperemia.

The causes of the hyperemia are not completely understood but two main groups of causes are usually considered. The intra arterial pressure-fall during ischemia seems important in removing the stimulus for contraction of the smooth muscles in the vessel wall. The arteries therefore dilate widely during arrest of the blood flow and permit a great initial flow at release. The latter stimulates the smooth muscles, and the great initial flow is rapidly reduced to the resting level. This myogenic concept was originally introduced by Bayliss (6) and was later strongly supported by the well-known studies of Folkow (14 15) The question is how the findings of the present report compare with this concept.

As demonstrated the main change of the hyperemia with advancing degrees of obliterative disease was that the initial part of it became reduced both absolutely and in relation to the remaining part.

This initial loss of flow could be a result of rigid muscle vessels having a less ability to react to the pressure fall. This is however unlikely because obliterative arteriosclerosis principally involves extra muscular arteries. Indeed there is no doubt that the muscle vessels were normal in the limbs with incomplete main artery stenosis. Yet their hyperemic response was typically changed.

It is more reasonable to consider the reduced local pressure in ischemic limbs as a significant factor During the arrest the net reduction of the pressure within the arteries becomes, in ischemia less than normal. This fact should be regarded as having the same effect as the "packing" of the arteries with blood cells during arrest (32) The pressure fall was thereby

counteracted and the ensuing hyperemia subnormal.

Moreover the stenotic lesion itself must be considered. With advancing degrees of obliteration, the amount of blood that can come through becomes more or less independent of any pressure fall during arrest. This is illustrated in this report by the case where there was acute failure of a Dacron graft and the collateral flow had not developed or could not develop. The hyperemia of this case was zero. A later operation showed that the vessels distal to the stenosis were patent. Of relevance to this are the remarkable experiments made by Blair et al. (8). By a suitable digital compression of the main artery they were able to reduce or even abolish the hyperemia.

It therefore appears that in being responsible for the local pressure fall as well as for the restricted inflow the obliterative lesion itself is the limiting factor for the hyperemia.

As the second group of causes for the hyperemic response several factors have been mentioned such as histamine, oxygen lack and metabolites (10, 12, 42, 43). The metabolite theory would require quantitative repayment of the incurred debt during arrest. In this respect the present findings in normal limbs might substantiate the theory. The incurred debt was more than repaid by the hyperemic flow. The mean repayment of the normal calf was 103 %, which compares favorably with that of 106 % of the normal hand (24) and with that of 109 % in the skeletal muscle of the dog (43).

However in ischemic limbs the repayment decreased with advancing degrees of arterial insufficiency. The prolongation of the hyperemia could not prevent a reduction of its total magnitude. This finding supports that of Dornhorst and Whelan (11) who obtained the same result in normal limbs by lowering their local effective blood pressure. Such a response is unlikely to occur if metabolites were mainly responsible for the hyperemia. In any case their removal can not be critically dependent on the actual rate of flow. If the removal of the metabolites occurs oxidatively it may be explained by an increased oxygen utilization during the hyperemia in ischemic limbs (30, 43).

The analysis of the hyperemia also adduced some results which need further comments. It appeared that an increased peripheral flow may be of two different types. It may be a vasodilatation and it may be an improvement of the flow. A certain care seems to be necessary in using these terms. In both dilatation and improvement there is an increase of the peak flow and the total excess flow. The only way by which the two conditions can be distinguished from each other is to evaluate the shape and the composition of the hyperemia. The peak flow alone is therefore an unsatisfactory index when either condition can occur. In addition any change of the peak flow may be missed as a result of technical errors. It therefore seems best to record also the first-minute flow of the hyperemia. If this flow value is changed, the relation between this flow and the flow throughout the remaining part of the hyperemia should be evaluated.

It follows that the kind of vasodilatation occurring in response to increasing periods of ischemia entails no improvement of the flow. It is only a consequence of a transient pressure fall and an accumulation of metabolites distal to the stenosis. The tube is unaltered. An improvement of the flow requires a better hyperemic response than before to the

same time of ischemia. This can only be brought about by a recanalization of the main tube a by passing of the stenosis by collaterals and grafts, or an increase of the systemic blood pressure.

Finally a very delicate problem should be considered in some detail. Is it possible from the experiments of this report to make any remarks concerning the effect of procedures aimed at decreasing the peripheral resistance? Is it reasonable to believe that sympathectomy and vasodilating agents in reducing the tone of the muscle vessels are able to improve the true blood supply? It is clear that an increased pressure fall during arrest would cause an increase of the hyperemia. But *the question is whether or not such a pressure fall* would ensue from the above measures.

A permanent reduction of the tone of the muscle vessels would in the first place entail a further pressure fall distal to the main artery stenosis. The pressure gradient over the stenosis would rise, the collaterals would dilate and more blood flow result. The blood flow at rest would increase, but this is not consistent with an improvement of the capacity of the circulation According to the present report this requires an increase of the *hyperemia especially its first stage.* In using the mentioned treatment the local effective pressure would be lower than before when the circulatory arrest was applied Consequently the net reduction of the local pressure during arrest would possibly be less than before and the ensuing hyperemia less. Based on the behavior of the hyperemia such treatment can therefore be of little use for the efficiency of the circulation With regard to the collaterals it is probable that any measures aimed at changing their tone would have *only a transient* effect, because the caliber of the collaterals seems to be regulated merely by the pressure gradient over the stenosis.

Summary

The calf blood flow at rest and following timed circulatory arrest has been studied by means of plethysmography The study was carried out in a series of normal limbs and limbs with intermittent claudication due to obliterative arteriosclerosis

The blood flow at rest was found to be of the same magnitude and range in both normal and ischemic limbs.

The hyperemic blood flow in response to timed circulatory arrest proved to be a *reliable index of the actual blood supply*

The use of increasing periods of arrest revealed the characteristic differences between the normal circulation and the circulation in peripheral obliterative disease. With advancing occlusive disease the hyperemia diminished so that the repayment decreased. There was especially a reduction of the flow in the first minute after release. The remaining part of the hyperemia became somewhat prolonged but not enough to compensate for the initial loss of flow The peak flow also diminished. The contrary changes took place when the circulation through ischemic limbs was improved by reconstructive surgery

In response to increasing periods of circulatory arrest the hyperemia underwent changes, which were also characteristic. The peak flow increased as did the total hyperemia. But its composition was also altered The first minute flow increased relatively less than the remaining part of the hyperemia the latter becoming protracted

It is discussed how the vasodilatation *following increasing periods of ischemia*

can be distinguished from an improvement of the circulation. Besides determining the peak flow and the hyperemia it is necessary to estimate the relationship between the first-minute flow and the flow during the rest of the hyperemia.

The use of the reactive hyperemia test in assessing the peripheral blood flow is evaluated and illustrated by examples of the hyperemic response at different stages of arterial insufficiency The corresponding changes of the hyperemia are discussed in the light of the present concept of the causes of this reaction.

The experiments provide evidence that measures aimed at decreasing the tone of the muscle vessels can hardly increase the hyperemic response.

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The Basophil Leukocytes in Patients with Bleeding Peptic Ulcer

By

Lennart Juhlin

Histamine is one of the most powerful stimulants of gastric hydrochloric acid secretion. There is strong evidence that supports the role of histamine as a chemo-stimulator of the parietal cell (3, 8 for ref.). The observation that there is no detectable change in concentration of histamine in blood after stimulation of gastric secretion by feeding, histamine infusions or vagal stimulation (1, 4, 9) contradicts this hypothesis. However, the methods for determining histamine may not be sufficiently sensitive for the detection of such minute changes.

Most of the circulating histamine in the bloodstream is carried by the basophil leukocytes. It was therefore thought that a study of the morphology and number of these cells in patients with peptic ulcer would be of interest.

Material

Studies were made in seventeen male patients, aged 20—82 years, admitted to the ward for acute gastric or duodenal bleeding with symptoms of haematemesis and/or melaena. They all gave a history of relapsing symptoms of gastric or duodenal ulcer of 2—10 years duration. They were kept in bed for at least as long as blood could be detected in the stools. On the day of admission to the hospital, they were often given ice-cream and a milk-rich, non-irritant ulcer diet on the following days. An antisecretory drug (Very1® Pharmacia, Uppsala) was given 3 times a day and an antacid (Novalucol® Astra, Sodertalje) was given several times a day when needed.

The patients' minimal haemoglobin values were between 7.6 and 12.2 g % (50—90 %) during the first days. The serum iron was 10—40 μg %. The iron deficiency was treated by daily intramuscular injections of 100 mg of an iron sorbitol citric acid complex (Jectofer® Astra, Södertälje) over a period of 10—15 days. The reticulocytes increased and the haemoglobin values returned to normal within the following weeks.

Radiological examinations of the stomach and duodenum were made on one of the first days after admission. The patient was in an upright position for a short time during this examination. If no ulcer was found, the origin of the bleeding was further looked for by X-ray examination of the lungs, oesophagus and the whole intestinal tract. This was complemented with rectoscopy, counting of thrombocytes, and bleeding and coagulation times. Nose and throat was carefully examined for source of bleeding.

Ulcers were found by X-ray examination in 8 cases. Their location was as follows: minor curvature of stomach, 3 cases, pyloric area, 2 cases, duodenum, 2 cases, stomach and duo-

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Table I The blood basophil differential in patients with verified peptic ulcer For comparison the values in 20 healthy subjects are given within the brackets The figures indicate the mean percentage of the different types of basophils

Class	Group A	Group B	Group C
	"Melted clumps of granules	Discrete med um granules	Small faint granules
1 Numerous intracellular	0 (2)	10(11)	6 (10)
2 Ring aggregation	2 (9)	5(12)	5 (6)
3 Surface extrusion	4 (7)	12 (6)	2 (1)
4 Extracellular spray	4 (4)	11 (5)	1 (1)
5 Massive release	2 (1)	7 (2)	1 (1)
6 Residual few	7 (7)	15 (7)	6 (6)

denum, 1 case. In the remaining 9 patients where an ulcer could not be verified radiologically the cause of the bleeding was probably due to superficial erosions in the mucosa. A deformation of the bulb was found in two of these patients.

The Weber test for faecal blood was negative 5—10 days after admission At this time, blood was taken for basophil and eosinophil leukocyte examinations A basophil count was made about one month later in some of the patients.

Methods

Counting and examination of basophils

The method for handling the basophils has been described elsewhere in detail (12) In principle, venous blood is withdrawn into a siliconized needle and syringe and forcibly ejected into a cold fixative (acetic acid 20, ethyl alcohol 60 chloroform 20) This destroys all of the erythrocytes, leaving only the leukocytes. The white cell suspension is filtered through a coarse membrane filter of cellulose (Cella 0) The cells remain on the filter where they are stained with toluidine blue. The filter is then dehydrated in ethyl alcohol, cleared in xylene and mounted on a microscope slide. It is then possible to study the morphology of the basophils, which appear as distinct cells with specific, dark metachromatic staining of the granules. The other white cells are pale blue with a faint greenish cast in the granules of the eosinophils.

Under a magnification of 640 × 20 or 40 consecutive basophils were classified into three major groups A, B and C, depending upon the size of the granules and the depth of the staining Furthermore, each group is subdivided into six classes (1 to 6) according to the number and location of the granules.

The percentage of basophils was estimated by counting the number of all the white cells seen while finding the 20 or 40 basophils. The total number of basophils has been estimated by counting the number of white cells/mm³ simultaneously

Eosinophils

The total number of eosinophils was counted in the manner described by Thorn et al. (14)

Results

1 In 8 patients with radiologically verified peptic ulcer the basophil leukocytes were reduced in numbers (mean value 14/mm 0.28 %) and they showed signs of increased degranulation Thus in the basophil differential the A_{1-3} cells had decreased and the B_{4-6} cells had increased ($P < 0.01$) when compared with normal subjects and patients with symptomatic but not verified bleeding peptic ulcer (table I) Five of the patients were examined 3—6 weeks later when they were in apparently good health. Their basophils at this time showed normal values.

2. In 9 patients with probable gastric bleeding but where no ulcer was found on X-ray examination, the number of basophils was normal (32/mm³ 0.54 %) Their basophil differential did not significantly differ from that of healthy subjects.

3 The individual values of the numbers of basophils and eosinophils appear in fig 1 No correlation was found between the numbers of the two cells. The haemoglobin values at the time of basophil examination varied between 50 and 71 % (7 6—10.8 g % mean 9.0 g %) in the patients with verified peptic ulcer and between 50 and 80 % (7.8—12.2 g %, mean 10.2 g %) in the other patients with gastro-intestinal bleeding.

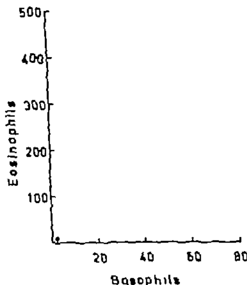


Fig. 1 Number of eosinophil and basophil leukocytes per mm³ blood in patients with bleeding peptic ulcer
● Patients with peptic ulcer verified by X-ray examination.
× Patients with intestinal bleeding without radiologically verified ulcer

Discussion

The mucosa and submucosa of the stomach contain an abundance of mast cells (10 13 for ref.) The mast cell, like the basophil leukocyte, has granules that contain both histamine and heparin. Degranulation of the mast cells has been observed in the gastric mucosa (5 10 11) and in various tissues (2) during treatment with ACTH and corticosteroids. Degranulation of the mast cells has been assumed to be associated with an increased content of histamine in the tissues. Kraft and Kirstner (7) therefore suggested that such a liberation of histamine could be a possible explanation for the increased hydrochloric acid secretion and incidence of peptic ulceration which some have observed during the treatment with ACTH and adrenal corticosteroids

The cause of the bleeding in our patients where no peptic ulcer could be seen on X-ray examination was probably due to a superficial erosion. The erosion is usually classified as an acute ulcer (6) In contrast to the severity of its clinical manifestation the acute bleeding ulcer presents a quite innocent appearance. It is usually only demonstrated after the stomach is opened by an exploratory incision. These patients usually showed normal basophil values. Their stress situation at the time of the investigation should be the same as in those with verified peptic ulcer Th slight numerical decrease of the circulating eosinophils was not significant Haemoglobin values in the two groups also did not differ significantly It is therefore less probable that the decrease in basophils in patients with radiologically verified peptic ulcer was due only to the acute stress situation caused by the bleeding

Fewer and more degranulated, basophils were found in the blood of patients with peptic ulcer verified by radiology

The degranulation and disappearance of the basophils in these patients might at least theoretically be taken as a sign of an increased endogenous histamine release. The released histamine could then cause increased hydrochloric acid secretion and peptic ulcer in a similar way as postulated for the mast cells (7) If such a mechanism is involved here, it could help to explain why these patients developed a peptic ulcer deep enough to be verified by radiology However the cause of the basopenia and degranulation in these patients cannot be explained at present and the matter needs further study

Summary

In 17 patients with symptoms of bleeding peptic ulcer the number and type of the circulating basophil leukocytes was estimated In 8 patients where the ulcer could be verified by radiology the basophils were decreased (mean value 14/mm³) and showed signs of degranulation. The basophil values returned to normal after 3—6 weeks of treatment. In the remaining 9 patients, no source of the bleeding could be demonstrated by X ray examinations. The bleeding in these cases was probably due to a superficial erosion of the mucosa. These patients had a mean basophil differential and count which did not differ from that found in healthy subjects. The total numbers of eosinophils and the haemoglobin values were nearly the same in both groups. The possibility that the basophils are endogenous producers of histamine and thus act as factors in the aetiology of peptic ulcer has been discussed

Acknowledgements

The author wishes to thank Mrs. Irma Kihlman for valuable technical assistance. The investigation was supported by grants from the Swedish Medical Research Council.

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Reticulocytosis in Congestive Heart Failure

By

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The production of red cells by the bone marrow and their release into the circulation is a series of integrated events designed primarily for a simple and vital purpose to transport oxygen from the lungs to the tissues. The exact mechanism of this regulation is little known. It has been suggested that a local hypoxia in the bone marrow governs the extent of hematopoiesis, but this assumption has never been confirmed in animal experiments. The prevailing theory is that the bone marrow is stimulated by various, so far unknown, intermediaries (13). Seip (11) and other investigators believe that there is a center in the brain stem regulating the erythropoiesis, and that its activity is dependent on the oxygen content of the blood

In normal individuals passing from low to high altitudes, the number of reticulocytes will rise after a few hours and remain elevated for some weeks. Under pathological conditions, for instance in chronic pulmonary diseases with constantly reduced oxygen tension in the arterial blood polycythemia develops. In experimentally induced hypoxemia there is a rapid release of reticulocytes from the bone marrow In animal experiments Nordström (9) obtained a mean increase of reticulocytes of 20 per cent by reducing the oxygen saturation to below 62 per cent. The rise occurred in the course of two hours and was most rapid in severe cases of hypoxemia.

In heart failure Ehrström (1) observed a reticulocytosis that subsided when the failure was compensated. Hedlund (5) has studied the number of reticulocytes in cardiac failure. It reached its peak when the failure was most marked and fell slowly to normal values when the failure subsided during treatment. On the other hand, it remained permanently increased if the patient did not recover In smears of capillary blood from patients with cardiac failure there is often a more or less pronounced polychromasia. Nucleated red cells may be present in grave

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The degranulation and disappearance of the basophils in these patients might at least theoretically be taken as a sign of an increased endogenous histamine release. The released histamine could then cause increased hydrochloric acid secretion and peptic ulcer in a similar way as postulated for the mast cells (7) If such a mechanism is involved here, it could help to explain why these patients developed a peptic ulcer deep enough to be verified by radiology However the cause of the basopenia and degranulation in these patients cannot be explained at present, and the matter needs further study

Summary

In 17 patients with symptoms of bleeding peptic ulcer the number and type of the circulating basophil leukocytes was estimated In 8 patients where the ulcer could be verified by radiology the basophils were decreased (mean value 14/mm³) and showed signs of degranulation. The basophil values returned to normal after 3—6 weeks of treatment. In the remaining 9 patients, no source of the bleeding could be demonstrated by X ray examinations The bleeding in these cases was probably due to a superficial erosion of the mucosa. These patients had a mean basophil differential and count which did not differ from that found in healthy subjects. The total numbers of eosinophils and the haemoglobin values were nearly the same in both groups. The possibility that the basophils are endogenous producers of histamine and thus act as factors in the aetiology of peptic ulcer has been discussed

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ble I Reticulocyte values. Range of variation brackets

oup	10 normal adults (/1000)	33 patients with cong. heart failure (/1000)
	0.1 (0—1)	0.2 (0—1.5)
I	0.5 (0—1)	1.9 (0—8.5)
II	2.6 (1—6)	7.9 (1—20)
IV	11.0 (7—14)	22 (11—40)
Total	14 (8—19)	32 (12—64)
I + II + III / IV	20/80	32/68

enumerator and denominator are expressed in per cent of total number of reticulocytes.

Blood was taken anaerobically from the femoral artery and examined immediately after the puncture. pH was determined at 37° C using the Sanz micro-electrode and the Metrohm pH meter. Analysis of the oxygen saturation was done by spectrophotometry with special cuvettes (2). Total carbon dioxide content was determined using the Kopp-Natelson microgasometer (5). On the basis of pH and total CO_2 the carbon dioxide tension has been calculated using the Henderson-Hasselbach equation. Reticulocyte counts and blood gas analyses were done by the author.

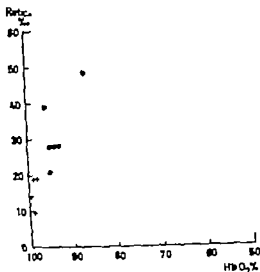


Fig. 2. Relationship between arterial oxygen saturation and number of reticulocytes.

○ rheum. or arterioscler. vit.
● cor pulm.
+ normals.

Results

The reticulocyte values in 10 normal individuals and 33 patients with cardiac failure are shown in table I. In the normal group the number of reticulocytes ranged from 8 to 19 per thousand averaging 14 per thousand. In the patient group the reticulocytes ranged from 12 to 64 per thousand, veraging 32 per thousand. In cardiac failure differential count revealed a relatively larger increase of the youngest cells, a so-called shift to the left.

The relation between oxygen saturation and number of reticulocytes is apparent from fig. 2. In the 10 normal individuals the oxygen saturation was 97—100 per cent, whereas the oxygen saturation in the patient group was 55—96 per cent. The patients with cor pulmonale presented the lowest values. According to degree of hypoxia, the patients can be divided into three groups (table II).

I. Slightly reduced or borderline values with an oxygen saturation of 90 to 96 per cent. Number of reticulocytes ranged from 12 to 45 per thousand averaging 27 per thousand. The youngest groups constituted 24 per cent.

II. Moderately reduced oxygen saturation, 75 to 89 per cent. Number of reticulocytes ranged from 24 to 52 per thousand, averaging 37 per thousand of which the youngest groups constituted 34 per cent.

III. Markedly educed oxygen saturation, 55 to 74 per cent. Number of

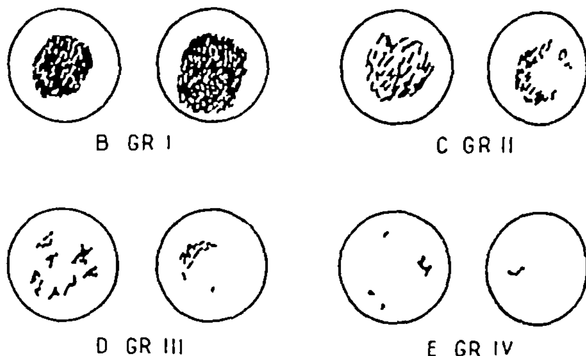


Fig 1 Diagram illustrating the groups of reticulocytes.

cases. Schwartz and Stansburry (10) pointed out that the presence of nucleated red cells in smears of capillary blood indicates a poor prognosis 66 of their 195 patients with such changes died shortly after the examination. Kvalvik (6) observed that the hematologic changes subsided with improvement of the heart failure. He commented that demonstration of reticulocytosis, polychromasia, and nucleated red cells may bring to mind a hemolytic syndrome at least when the changes are marked

Normal individuals and patients with cardiac failure have been investigated to find out whether there exists a relationship between the reticulocytosis and the oxygen saturation of arterial blood The influence of disturbances in the acid base balance is also studied. Differential counting of the reticulocytes according to degree of maturity is used to indicate whether a relative increase of the youngest cells is present.

Material and methods

Thirty three patients with severe cardiac failure have been examined Five patients had cor pulmonale, all the others arteriosclerotic or rheumatic heart disease. The examinations were done when the patient's condition had remained stationary for some hours. None of the patients examined had anemia or other diseases that might influence the results.

Reticulocyte counting and blood-gas analysis have also been carried out in ten normal individuals.

The reticulocytes were stained with brilliant cresyl blue using a modification of the Holböll method (11) Each count included 2,500 red cells. According to size and extent of the reticulum these cells can be divided into 4 groups (4 11 12)

The youngest cells have a high content of reticulum (fig 1) With increasing age and maturity the reticulum diminishes and the cell approaches the mature erythrocyte. When a shift to left is present there is a relative increase of the youngest cells, represented by groups I, II and III The ratio between the younger and the most mature reticulocytes in group IV indicates a shift to left. The

of reticulocytes. This shows that in cardiac failure the youngest cells increase the most.

Hedlund (3) examined the erythropoiesis in cardiac failure. He found an intensified erythropoiesis resulting in an elevated total red cell volume. As the number of reticulocytes is increased more than the elevated total red cell volume indicates, a shortened survival of the erythrocytes is possible during cardiac decompensation. Ehrström (1) observed increased total excretion of urobilin when the symptoms of insufficiency were subsiding. He concluded that this is a manifestation of increased hemolysis.

In preliminary investigations I have found a markedly shortened survival of the red cells in cardiac failure, and further investigations are going on.

Conclusion

The present investigations show rising reticulocytosis with increasing hypoxemia, but no absolute correlation. Perhaps oxygen saturation in the hypothetical brain center that is influenced by hypoxemia is different from that of the femoral artery. There was no relation between reticulocytosis and changes in carbon-dioxide tension or pH.

Summary

Reticulocyte counting including a differential count, the oxygen saturation, carbon-dioxide tension and pH of arterial blood were studied in 33 patients with cardiac failure.

The investigations show rising reticulocytosis and shift to the left with increasing hypoxemia. There was no relation between reticulocytosis and changes in carbon-dioxide tension or pH.

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Table II Arterial oxygen saturation and number of reticulocytes in 10 normal adults and 25 patients with congestive heart failure Range of variation in brackets

No.	O_2-saturation (%)	Reticulocytes (/1000)	Groups I+II+III / IV
10 normals	97–100	14 (8–19)	20/80
12 patients	90– 96	27 (12–45)	24/76
8 patients	75– 89	37 (24–52	34/66
5 patients	55– 74	37 (27–51)	37/63

Table III Carbon-dioxide tension in arterial blood and number of reticulocytes in patients with cardiac failure

pCO (mm Hg)	No. of cases	Reticulocytes (/1000)
27–34	9	36 (27–52)
35–45	11	26 (14–48)
46–90	5	38 (33–51)

reticulocytes ranged from 27 to 51 per thousand averaging 37 per thousand The youngest constituted 37 per cent.

The study shows that the two groups of patients with the lowest oxygen saturation also present the most marked reticulocytosis and shift to the left. There is no overlapping between these values and those for the normal individuals since the lowest number for patients with moderately and highly reduced oxygen saturation is 24 per thousand whereas the highest number for normal individuals is 20 per thousand

Table *III* shows the relation between carbon dioxide tension in arterial blood and number of reticulocytes. The five patients with cor pulmonale had a markedly increased carbon dioxide tension and they all belong to the group with the highly reduced oxygen saturation In all other types of cardiac failure the carbon-dioxide tension was slightly reduced or normal. Nearly half of the patients with heart failure presented a carbon-dioxide tension within the normal range i. e. 35—45 mm Hg Mean number of reticulocytes in patients with increased or reduced carbon-dioxide tension was *higher than* in cases where the carbon-dioxide tension was normal. This shows that the reticulocytosis is independent of this factor

It is noteworthy that there was no definite correlation between changes in acid base balance and oxygen saturation and the clinical picture It is well known that no relation exists between degree of cyanosis and oxygen saturation nor between dyspnea and carbon-dioxide tension

In 22 patients pH lay within the normal range i e. 7.35—7 45 Only two patients showed slightly increased values (7 45—7 50) One patient, with severe cor pulmonale had a low value of 7 18

Discussion

The normal values for reticulocytes vary somewhat and are dependent upon staining method and counting technique. Seip (11) found an average of 15 7 per thousand using the same staining method as in the present study Differential counting of reticulocytes will always, to some extent, be arbitrary The estimation of shift to the left can therefore only be regarded as valid when *all* counts are done by one and the same person. In the normal group of this study the youngest cells constituted an average of 20 per cent and in patients with cardiac failure 32 per cent of the total number

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Alpha-Methyldopa in Arterial Hypertension

Clinical, Renal and Hemodynamic Studies

by

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A great number of different drugs are available for routine clinical use in the treatment of arterial hypertension. Regarding their modes of action they may be divided into only five or six well defined groups. Each group contains several drugs with minor variations in structure, dosage etc., which from the clinical point of view are of no importance. Appearance of new drugs within these groups usually does not add anything to the therapeutic regimen.

The development of alpha-methyldopa signifies the creation of new drug that may introd ce new pharmacological principle n the treatment of arterial hypertension.

Alpha-methyldopa (= α-methyl-3 4-dihydroxy DL-phenylalanine) is the methylated analogue of dopa (fig 1) It was synthesized by Stein et al. (24) in 1953 The substance was shown to have an inhibitory effect both in vitro and in vivo on l. a. the decarboxylase promoting the reaction dopa to dopamine, thus to a great extent blocking the synthesis of norepinephrine (8 23) It also possessed an inhibitory action on the production of serotonin, which led Sjoerdsma et al. (22) to use it in carcinoid patients. They noted a fall in blood pressure in several patients. The possible usefulness of alpha-methyldopa for treatment of arterial hypertension was therefore studied (17) They later found the hypotensive effect to be limited to the l-isomer (11)

Since the original reports of Sjoerdsma et al. a great deal of interest has been paid to the effectiveness of alpha-methyl dopa in the treatment of arterial hypertension, and several reports from different countries have appeared.

For more than two years the l isomer of alpha-methyldopa has been submitted to clinical trial at Sahlgren's hospital. The present report will deal with the experiences of long term treatment in arterial hypertension together with a comment on the effects on renal and systemic hemodynamics in hypertensive in-patients.

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administration of 0.75—2.0 g of AMD for 7—16 days. For details see Sannerstedt et al. (20). The work load was in eight cases 600 kpm/min., in the tw others 200 and 400 kpm/min. respectively. Intraarterial blood pressure recordings were obtained from a brachial artery. Cardiac output was deter mined using a dye dilution technique.

B The patients were studied before (C) and after 6—19 days of treatment with AMD, up to 2.0 g daily (A). The doses were varied according to the hypotensive response, both supine and standing. N side-effects were noted in this group of patients. Indwelling arterial catheters were used for blood pressure recordings, infusions and blood sampling. The renal veins were catheterized in six patients from the femoral ein using standard techniques.

Glomerular filtration rate nd renal plasma flow were estimated as the clearances of inulin and para-aminohippurate respectively using constant infusion techniques and mid-period arterial blood samples. Urine was collected during 3—5 successive clearance periods, each of 20 min., through an indwelling bladder catheter.

Cardiac output was determined twice during the middle part of the clearance periods using the indicator dilution technique. Blood pressures were repeatedly recorded by a strain gauge electrical manometer (Elema) in each period. All values are averaged in table IV giving one value for the control and one for the treatment period.

Details of the procedure and laboratory analyses have been reported earlier (4).

Renal vascular resistance was calculated using the figures for PAH-clearance which represents renal plasma flow as there was no change in the renal extraction of PAH in the five subjects where this was determined before and after AMD.

C. I all but tw cases treatment with AMD was started during the hospital stay (table II). The drug was administered in capsules or tablets containing 0.25 g of the l-isomer. The initial dose aried between 0.125 and 2.0 g per da usually 0.75—1.0 g divided in 3—4 doses per day. The dose was increased, if necessary, by 0.25 g at an interval of 2—3 days during hospital stay and at least one week during ambulatory treatment. Doses of more than 2.0 g per day have not been used.

Table II. C. Effect on arterial blood pressure level. Performance of treatment

	No. of pat.
Treatment started	
During hospital stay	31
On hospital dismissal	2
Initial dose/day	
≦0.5 g	9
0.75—1.0 g	16
1.5 g	7
2.0 g	1
Highest dose day	
<1.0 g	9
1.0—1.75 g	18
2.0 g	6
Maintenance dose day	
0.5 g	6
0.75—1.0 g	17
1.25—2.0 g	3
α-methyldopa given	
Alone	8
In combination with saluretic drugs	17
In combination with saluretic drugs + hydralazines	5
In combination with saluretic drugs + hydralazines + gangl. or symp. blocking drugs	3
Duration of treatment	
< 2 months	6
2— 6 months	12
7— 12 months	11
13—20 months.	4

In the 26 patients where maintenance dose could be arrived at, it varied between 0.5 and 2.0 g, most often 0.75—1.0 g per day.

In eight subjects AMD was the only drug given. In the others it was combined with various hypotensive agents, usually a saluretic.

Fifteen patients have been under treatment with AMD for more than six months.

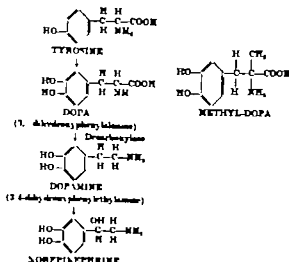


Fig 1

Material

α-methyldopa (AMD) has been given orally to a total of more than 50 patients with arterial hypertension of different causes and stages. Three different types of study have been used.

A. CARDIOVASCULAR EFFECTS

In ten patients the influence of AMD on cardiac output and blood pressure at rest and during exercise was studied Detailed results from six of these patients have been published elsewhere (20)

B. INFLUENCE ON RENAL FUNCTION

In eleven hospitalized patients with mild to moderate hypertension the influence of AMD on renal blood flow and glomerular filtration rate was studied under basal conditions in the recumbent position. In three of these patients hypertension was known for one year or more (A. H., K. J and H E.) and in the remaining patients recently diagnosed. Renal arteriograms performed in all patients did not reveal any signs of renal artery stenosis. Catechol amine excretion was normal

No hypertensive drugs had been given for several weeks before the control study but the patients had been hospitalized and investigated for 3—7 days before this study

C. EFFECT ON ARTERIAL BLOOD PRESSURE LEVEL

Thirty-three patients — 17 males and 16 females aged 24—72 years — were followed up to 20 months (table I) Six patients were in

Table I C. Effect on arterial blood pressure level. Clinical data. Thirty-three patients (17 males and 16 females)

	No. of pat.
Age (24—72 years)	
<50 years	9
50—60 years	21
>60 years	3
Group according to Keith-Wagener	
I—II	27
III—IV	6
Clinical diagnosis	
Ess. hyp.	20
Hypercholesterolemia + hyp.	2
Hyp. cardiovasc. disease	3
Chron. pyelonephrit. + hyp.	4
Renal art. stenosis + hyp.	3
Diabetes + hyp.	1
Treatment immediately before α-methyldopa	
None	15
Saluretic drugs	4
Saluretic drugs + hydralazines	2
Saluretic drugs + hydralazines + gangl. or symp. blocking drugs	7
Other forms of combined treatment	5

group III—IV according to Keith-Wagener Twenty patients were diagnosed as essential hypertension. Among the others there were three cases of renal artery stenosis, one of which had previously been operated upon. Fifteen patients were previously not treated with hypotensive agents. In the other cases side effects or inadequate blood pressure adjustment justified revision of treatment usually one or more of the previously used drugs were kept but in reduced dosage. These patients often represented difficult therapeutic problems with sustained high levels of blood pressure.

Methods and performance

A The patients were studied at rest and during graded exercise in the sitting position on a bicycle ergometer before and after

able II B. Influence of α-methyldopa on renal function Studies in eleven patients with mild to moderate pertensive disease before and during treatment

Case, sex, age	Study	Heart rate (beats/min)	Brachial artery B. P (mm Hg) Systolic/Diastolic	Mean	Cardiac output (l/min)	Total peripheral vascular resistance (units)	Inulin clearance (ml/min)	Para-aminohippurate clearance (ml/min)	Extraction of para-aminohippurate in %	Estimated renal blood flow (ml/min)	Total renal vascular resistance (units)	Renal fraction of cardiac output in %
A. H.	C	74	224/120	160	5.20	30.8	117	327	89	595	269	11 4
♂ 34	A	62	199/111	146	4.97	29.4	109	388	89	654	223	13.2
S. M.	C	87	173/110	143	6.41	22.3	147	682	87	1,263	113	19 7
♂ 51	A	57	172/92	129	5.34	24.2	132	733	82	1,308	99	24.5
K. J	C	73	192/95	135	4.97	27.2	87	437	91	795	170	16.0
♂ 50	A	76	165/94	125	4.46	28.0	103	432	89	837	149	18 0
A. M.	C	69	221/96	149	7.77	19.2	150	617	69	1 102	135	14.2
♂ 58	A	67	209/112	135	—	—	142	691	—	1,234	126	—
L. T	C	61	187/90	125	5.08	24 6	78	304	91	582	215	11.5
♂ 44	A	60	150/71	103	3.61	28.5	62	406	91	677	152	18.8
O. A	C	48	170/78	115	5.62	20.5	113	560	87	966	119	17.2
♂ 32	A	44	134/61	89	4.70	18.9	111	634	84	1,128	79	24.0
O. L.	C	54	203 97	142	7.07	20.1	127	736	—	1,132	123	16.0
♂ 43	A	49	198 99	144	7.97	18.1	114	726	—	1,134	127	14.2
H. E.	C	44	229 78	121	—	—	75	380	—	655	185	—
♂ 62	A	41	211 72	112	—	—	79	412		644	174	—
M. G.	C	83	259 120	178	—	—	83	441	—	735	242	—
♂ 44	A	61	177 87	122	—	—	76	444	—	634	192	—
O. H	C	62	142 58	69	—	—	124	661	—	1 140	78	—
50	A	49	147 52	83		—	98	488	—	800	104	—
H. A.	C	73	174 77	113	—	—	86	494	—	810	140	—
56	A	63	177 82	114	—	—	82	598	—	865	118	—

C = control study A = during treatment with α-methyldopa.

output determinations as well as the calculated vascular resistances for the eleven patients. Fig 2 shows in graphic form the results from representative case.

Under the experimental conditions used only six of the eleven patients had a diastolic pressure above 90 mm Hg during the control study Four patients had elevated systolic pressures whereas the remaining was essentially normotensive. Morning blood pressures taken in the ward revealed, however persistent diastolic hypertension in all patients except one (O H.) at the time of the control study

A slight to moderate fall in rterial mean pressure (10—56 mm Hg) was

Table III A. Cardiovascular effects of α-methyldopa. Studies on 10 hospitalized patients with essential hypertension

	Daily administration of 0.75—2.0 g α-methyldopa orally for 7—16 days	
	Changes *at rest* in the sitting position (mean values and range)	Changes *during exercise* in the sitting position upon a bicycle ergometer (mean values and range)
Heart rat (beats/min)	– ±0 (−20 to + 19)	↓ −15 (−35 to + 8)
Systolic brachial artery B. P (mm Hg)	↓ −29 (−54 to + 8)	↓ −56 (−87 to + 2)
Diastolic brachial artery B. P (mm Hg)	↓ −12 (−30 to ± 0)	↓ −19 (−39 to − 2)
Mean brachial artery B. P (mm Hg)	↓ −18 (−37 to − 5)	↓ −30 (−59 to − 2)
Cardiac output (l/min)	– −0.1 (−1.8 to +1.2)	– +0.2 (−2.6 to +2.8)
Stroke volume (ml/beat)	– −2 (−25 to + 22)	↑ +10 (−25 to + 24)

The treatment with AMD always started after several days of hospital rest, usually 7—10 days, in order to achieve a good basal blood pressure level. Auscultatory blood pressures in the recumbent and standing positions were taken every morning. The average of the last three morning recordings of blood pressure has been used as an expression for the basal blood pressure level. This pretreatment value has been compared with the average of the last three morning recordings of blood pressure after about two weeks of treatment in the hospital. The morning recordings were chosen for practical reasons. It is well recognized that these values will not reflect the diurnal variations, as it is in the morning that all neurogenic blocking drugs have their greatest effect on blood pressure.

After dismissal from the hospital the patients were seen regularly and auscultatory blood pressures were recorded in the lying and standing positions. Doses were adjusted to achieve a diastolic pressure in the standing position of less than 100 mm Hg. An expression for the ambulatory blood pressure level has been derived by taking the average of the latest three ambulatory blood pressure recordings. The ambulatory readings were usually not done in the morning and cannot logically be quite comparable to the hospital recordings. A lowering of the afternoon blood pressure will, however, reflect a greater therapeutic effect.

Results

A. CARDIOVASCULAR EFFECTS

The effect of AMD is presented in table III as mean changes for the whole group of ten hypertensive patients.

There was no change in resting heart rate, while it was lower during exercise. Mean arterial blood pressure at rest was lower in all subjects on an average 18 mm Hg. The decrease in systolic pressure was about twice that of the diastolic. During exercise the reduction of mean arterial blood pressure was still more marked — on an average 30 mm Hg — with quite normalized blood pressure reaction to work in several cases. No tendency to post-exercise hypotension was observed.

Cardiac output did not show any consistent change either at rest or during exercise. The blood pressure reduction was thus achieved by a lowered total systemic resistance. The stroke volume at rest was on the whole unchanged but increased during exercise by an average of 10 ml.

B. INFLUENCE ON RENAL FUNCTION

Table IV contains the mean data for renal clearances, blood pressures, cardiac

Table V C. Effect of α-methyldopa on blood pressure level during hospital stay. Mean values and ranges for 15 previously untreated patients

	Lying	Standing
Before α-methyldopa		
B.P. (mm Hg) on admission	194/121 (140–240/100–145)	187/121 (145–220/95–140)
Average of last 3 B.P. recordings in the morning during pre-treatment hospital stay (av. 10 days)	172/103 (140–207/83–120)	161/104 (117–190/82–122)
During α-methyldopa		
Average of last 3 B.P. recordings in the morning after 13 days (av. value) of treatment in the hospital	152/95 (118–187/72–117)	136/90 (97–178/67–122)
Lowest recorded B.P.	136/87 (110–170/70–110)	117/80 (80–145/60–115)
Dose of α-methyldopa/day	1.3 g (0.5–2.0 g)	

Table VI. C. Effect of α-methyldopa on blood pressure level during hospital stay. Mean values and ranges for 11 previously treated patients

	Lying	Standing
Before α-methyldopa		
B.P. (mm Hg) on admission	223/131 (190–260/105–160)	195/128 (140–270/100–163)
Average of last 3 B.P. recordings in the morning during pre-treatment hospital stay (av. 7 days)	185/109 (143–220/93–122)	170/107 (130–200/87–127)
During α-methyldopa		
Average of last 3 B.P. recordings in the morning after 12 days (av. value) of treatment in the hospital	167/97 (128–205/80–112)	133/89 (112–160/68–105)
Lowest recorded B.P.	149/87 (120–190/70–100)	118/82 (70–145/40–105)
Dose of α-methyldopa/day	0.9 g (0.5–1.5 g) in combination with various other hypotensive agents	

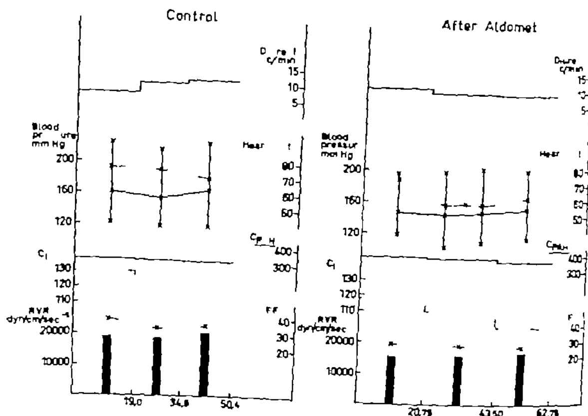


Fig. 2. B. Influence of alpha-methyldopa on renal function. Results from studies in a male with essential hypertension before and during treatment.

C_{In} = inulin clearance (ml/min.)
C_{PAH} = para aminohippurat clearance (ml/min.)
RVR = renal vascular resistance.
FF = filtration fraction.

noted in four of the six patients with elevated diastolic pressures following AMD administration.

Of the patients with diastolic pressures below 90 mm Hg at control study three (L. T., O. V. and H. E.) showed decrease in mean pressures after AMD.

Venous blood pressure as measured by renal vein catheterization did not change significantly after AMD in the five patients studied. A slight reduction in heart rate was observed in six patients after AMD, the other five being almost unchanged.

Cardiac output decreased after AMD in five of six patients studied. The calculated peripheral vascular resistance remained almost unchanged in each case.

Glomerular filtration rate and renal plasma flow did not consistently change for the whole group. However, five of the seven patients who showed a blood pressure decrease after AMD concomitantly increased their renal blood flow. In these seven patients the calculated renal vascular resistance decreased by an average of 18.7 per cent that proved to be statistically significant ($p < 0.01$). The renal frac

As a rule the hypotensive effect manifested itself within one or two days. In four subjects there was no appreciable effect on the blood pressure level. In those cases where the blood pressure was followed after withdrawal of AMD the initial blood pressure level was generally reached within two days in three patients, however the hypotensive effect remained up to five days.

In eleven previously treated patients the average blood pressure on admission was 223/131 and 195/128 mm Hg in the lying and standing positions respectively (table VI) After the pre-treatment hospitalization period the corresponding figures were 189/109 and 170/107 mm Hg respectively This basal pre-treatment value was lowered after addition of 0 9 g AMD daily to 167/97 and 153/89 mm Hg respectively The lowest recorded blood pressure during the period was on an average 149/87 and 118/82 mm Hg respectively The decrease in systolic pressures was, as in the previous group about twice that of the diastolic, and the blood pressure level was lowered both in the standing and in the lying positions.

EFFECT ON ARTERIAL BLOOD-PRESSURE LEVEL DURING LONG-TERM TREATMENT

In eight previously untreated patients (table VII) the average initial basal level of blood pressure during the pre-treatment hospital stay was 170/101 mm Hg in the recumbent and 160/103 mm Hg in the erect position. After treatment in the hospital the corresponding figures were 152/97 and 131/93 mm Hg respectively The daily dose of AMD was 1 1 g (average value)

After ambulatory treatment with AMD in a daily dose of 0.8 g for six months the average blood pressure level had increased to 180/105 and 157/102 mm Hg respectively in the both positions. The ambulatory blood pressure level was, however still significantly lower than the blood pressure level on admission and comparable to that obtained with optimal external conditions during hospital rest.

In 17 previously treated patients the initial basal blood pressure during pre-treatment hospital stay was 191/106 and 171/103 mm Hg respectively in the lying and standing positions (table VIII) With an added daily dose of 0.9 g AMD average blood pressure levels of 171/99 and 134/90 mm Hg respectively were reached.

After ambulatory treatment with the same amount of AMD for nine months the average blood pressure level had increased to 192/110 and 167/108 mm Hg respectively As in the previously untreated group the blood pressure level during ambulatory treatment was about equal to that obtained with optimal external conditions during hospital rest and significantly lower than that on hospital admission.

Several patients had previously been under complex treatment with three different drugs and with often severe side effects. During AMD treatment it was possible to maintain or even improve the blood pressure adjustment with significantly fewer side effects (table IX)

In many cases in both groups the doses of AMD had to be successively increased after dismissal from the hospital if the attained hypotensive effect was to be maintained (7 21) Frank development of tolerance was observed in at least three patients, in one subject already after 3—4 weeks of treatment. These therapeutic failures are listed in table IX.

Table VII C. Effect of α methyldopa on blood pressure level during ambulatory treatment Mean values and ranges for 8 previously untreated patients

	Lying	Standing
Before α-methyldopa		
B. P (mm Hg) on hospital admission	208/120 (160–260/100–130)	186/119 (160–200/100–130)
Average of last 3 B. P recordings in the morning during pre-treatment hospital stay (av. 10 days)	170/101 (143–207/83–115)	160/103 (117–173/82–122)
During α-methyldopa		
Average of last 3 B. P recordings in the morning after 10 days (av value) of treatment in the hospital	152/97 (127–170/85–117)	131/93 (112–153/82–122) (7 patients)
Daily dose of α-methyldopa during hospital stay	1 1 g (0 75–1.5 g) in 2 patients in combination with saluretics	
Average of latest 3 B. P recordings after 6 months (av value) of ambulatory treatment	180/105 (153–210/97–117)	157/102 (128–187/90–115)
Daily dose of α-methyldopa during ambulatory treatment	0.8 g (0.5–1.5 g) in 7 patients in combination with saluretics	

tion of cardiac output increased in five of the six patients studied The renal extraction of para aminohippurate was uninfluenced by AMD The filtration fraction was unchanged or slightly decreased.

C. EFFECT ON ARTERIAL BLOOD PRESSURE LEVEL DURING HOSPITAL STAY

In 15 previously untreated patients with an average blood pressure on admission of 194/121 mm Hg in the recumbent and 187/121 mm Hg in the erect position (table V) the blood pressure decreased during the pre-treatment period to 172/105 and 161/104 mm Hg respectively

After treatment for 13 days with AMD alone in a daily dose of 1.3 g there was a further decrease of the blood pressure to 152/95 and 136/90 mm Hg respectively in the recumbent and erect positions. The average of the lowest recorded blood pressures during the treatment period was 136/87 and 117/80 mm Hg respectively

The decrease in systolic pressure was about twice that of the diastolic. There was a significant drop in both systolic and diastolic pressures both in the standing and in the lying positions. A tendency toward orthostatic reactions was present in many cases. Systolic pressures in the erect position of 100 mg Hg or less were observed in five subjects at some point during the treatment period

Table IX. Some data on the therapeutic value of α-methyldopa

Pat. Sex Age	Group acc. to Keith-Wagener	Previous treatment	Side effects	B. P	Present treatment	Side effects	B. P	Comments
A. Successful change of previously complex treatment								
H. O. ♀ 58	II	Hy-chl. + hydral. + guan.	Headache+++	260/140 200/130	AMD + hy-chl.	0	190/108 158/93	–
I. S. ♂ 51	II	Res. + hydral. + pentol.	Impotence+++ Headache++ Tachycardia++	200/115 140/110	AMD + hy-chl.	0	175/107 147/108	–
A. P. 52	II	Chl. + hydral. + guan.	0	193/120 190/115	AMD + hy-chl.	0	172/98 150/100	–
E. W. 62	II	Chl. + hydral. + guan.	Tiredness+++ Orthostatism++	190/110 170/110	AMD + hy-chl.	0	187/110 167/105	–
V. A. ♂ 51	III	Hy-chl. + hydral. + guan.	Tiredness++ Dry mouth++	220/103 170/100	AMD + hy-chl. + hydral.	0	183/108 177/110	–
D. L. 56	IV	Res. + chl. + hydral. + guan.	Orthostatism+++	280/100 180/90	AMD + hy-chl. + hydral.	0	215/108 203/107	–
M. C. 57	IV	Chl. + hydral. + guan.	Vertigo+++ Orthostatism+++	240/120 210/125	AMD + chl. + hydral.	Vertigo ++	217/115 187/103	–
B. Therapeutic failures during long-term treatment								
E. H. 55	I	Chl.	0	240/160 210/160	AMD + chl.	0	210/145 190/155	Rapid develop. of tolerance
V. A. ♂ 42	III	Chl. + hydral. + guan.	Tiredness+++ Muscular Weakness+++ Orthostatism++	170/130 160/130	AMD + chl. + hydral. + guan.	Tiredness +++	183/128 160/130	Rapid develop. of tolerance
K. C. 37	IV	Chl. + guan.	Nose stuffiness +++	193/120 180/115	AMD + chl.	0	190/115 157/117	Rapid develop. of tolerance

B. P. in the lying and standing positions on hospital admission.
Averages of latest three ambulatory B. P. recordings in the lying and standing positions.
AMD = α-methyldopa (0.5—2.0 g day); hydral. = hydralazine; chl. = chlorothiazide
pentol. = pentolinium; guan. = guanethidine; res. = reserpine; hy-chl. = hydrochlorothiazide.

Table VIII C. Effect of α-methyldopa on blood pressure level during ambulatory treatment. Mean values and ranges for 17 previously treated patients

	Lying	Standing
Before α-methyldopa		
B. P. (mm Hg) on hospital admission	223/126 (160–290/100–160)	198/125 (140–290/90–165)
Average of last 3 B. P. recordings in the morning during pre-treatment hospital stay (av. 8 days)	191/106 (140–230/83–133)	171/103 (128–223/70–127)
During α-methyldopa		
Average of last 3 B. P. recordings in the morning after 11 days (av. value) of treatment in the hospital	171/99 (128–230/85–112)	134/90 (112–160/68–107) (13 patients)
Daily dose of α-methyldopa during hospital stay	0.9 g (0.5–1.5 g) in combination with various other hypotensive agents, usually saluretics	
Average of latest 3 B. P. recordings after 9 months (av. value) of ambulatory treatment	192/110 (142–228/95–148)	167/108 (137–203/93–148)
Daily dose of α-methyldopa during ambulatory treatment	0.9 g (0.5–2.0 g) in combination with various other hypotensive agents, usually saluretics	

Side effects

Side effects appeared relatively frequently but were often overcome by slight modifications of the treatment (table X).

Tiredness and sleepiness occurred regularly if the doses were kept high. Others have reported that the sedative effect will subside during continuous, unchanged medication (1, 7, 9, 15, 21); this was seen only occasionally in the present series. Persisting sedation led to withdrawal of the drug in three cases. Dose reduction, on the other hand, often brought about reduction or disappearance of this symptom. Reactive depression has not been observed.

Orthostatic symptoms were seen especially in the beginning of the trial but have not been a problem. They were avoided with cautious dosage and adequate intervals between dose increments. One female aged 72 with cerebral atherosclerosis became confused when showing marked orthostatic reactions, that led to withdrawal of the drug.

One male complained of impotence, another of failure of ejaculation. Few side effects from the gastrointestinal tract were observed.

Weight gain of 2—5 kg without any signs of heart decompensation was ob-

tendency at rest in the present series. During exercise, however a moderate reduction in heart rate was observed, resulting in an elevated stroke volume.

Of special interest is that the reduction of arterial blood pressure at rest not only persisted during exercise but was even still more marked without any unfavorable effect on cardiac performance. Thus several hypertensive patients showed a quite normal blood pressure reaction during exercise under treatment with AMD There was no tendency to post exercise hypotension. It is therefore unlikely that the hypotensive effect of AMD is mediated by a depressive action of AMD on myocardium.

The lack of any disadvantageous effect of AMD on glomerular filtration rate or renal plasma flow agrees with the findings of Cannon et al. (5) and Onesti et al. (18) Nor has any deterioration of renal function been observed during long-term treatment.

In the present series AMD was as effective in primary as in secondary hypertension. In patients with hypertension grade III—IV and high initial blood pressure it was often reduced. Cannon et al. (5) found several cases of severe and malignant hypertension to be sensitive to the drug On the other hand they found sympathectomized patients to be resistant to treatment with AMD which has, however not been apparent in the present series.

Treatment with AMD has in the present series proved to be especially effective in labile easily excitable and restless patients with high, fluctuating blood pressures. The moderate sedative action might be of special value in these patients.

AMD differs favorably from the ganglionic and sympatheuc blocking agents in that there is an appreciable blood pressure reduction not only in the standing position but also when the subjects are recumbent (3 5 7 9 11 15 21) However the blood pressure decrease is more pronounced in the erect position. Overdosage will provoke orthostatic reactions with its risks in elderly persons with rigid vessels. With adequate dosage it has been possible to avoid orthostatic reactions in most cases.

The decrease in systolic pressure is greater than that of the diastolic (5 15 18 21)

The main drawback of AMD has been the development of tolerance, necessitating successively increased doses. Doses of more than 1.5 g per day have often been accompanied by a persisting tiredness and sleepiness. A combination therapy with other hypotensive agents has therefore become standard the dose of AMD not exceeding 1.5—2.0 g per day This has also been advocated by others (1 21)

An initial dose of 0.25 g 2—3 times per day has been found to be suitable in most cases. During hospital stay the dose may if necessary be increased by 0.25 g per day every second to third day under continuous control of the blood pressure in both the lying and standing positions. The interval between dose increments during ambulatory treatment should be one week or more. If an adequate blood pressure response is not achieved by a daily dose of 1 0—1.5 g AMD a saluretic agent in a fixed dose might be added and if necessary also other hypotensive agents. In many cases it might be desirable to start the therapy with a combination of AMD and a saluretic agent.

Apart from sedation and orthostatic reactions AMD has been found to produce relatively few side-effects. Gillespie (10) reported two cases of febrile reactions in

Table X. Side effects of α methyldopa (mild side effects + moderate ++ and severe +++) Figures represent number of subjects

	+	++	+++
Sedation	2	6	4
Orthostatic troubles, dizziness	2	3	2
Cerebral deterioration (in combination with orthostatic reactions)	—	1	—
Obstipation	—	1	—
Impotence	—	—	1
Failure of ejaculation	—	1	—

served in some cases, as also reported by others (1 2, 3 9) No alterations in heart rhythm or rate attributable to AMD have been observed

No febrile reactions or clinical signs of toxic influences on liver or blood have been observed After more than five months of treatment a slight to moderate increase of the eosinophilic white cells was found in three patients four patients showed a moderate relative lymphocytosis. The significance of these findings remains obscure. Liver tests including GOT and GPT did not show any alterations that might be due to the treatment with AMD Renal function was unchanged

Discussion

It might be concluded from the results in the present series and from other reports (1 3 5 7 9 11 15 18 21 25) that alpha methyldopa (AMD) significantly reduces blood pressure in the hypertensive man. The exact mechanism is still not clarified.

Initially it was assumed that the main factor would be an inhibition of norepinephrine synthesis. It was, however observed in animals that there was no parallelism between inhibition of the decarboxylation process and lowering of the blood pressure, the latter appearing after a latent period of several hours (12) The excretion of norepinephrine or its metabolites in the urine is unaltered during AMD treatment, indicating that the inhibition of decarboxylation is not the primary hypotensive mechanism (5)

Animal experiments have shown that AMD interferes with the storage of norepinephrine in the brain and heart (6 14 19) The assumption that this would cause the hypotensive effect would explain the latent period mentioned above.

The present study on the cardiovascular effects of AMD indicates that the blood pressure is lowered by a reduction in total systemic resistance, while cardiac output is unchanged. This is in agreement with the findings of Kuschke et al. (16) who studied the acute systemic effect of AMD given intravenously Others (26) have found that intravenous administration lowers the cardiac output. Onesti et al. (18) found a moderate decrease of both cardiac output and peripheral resistance after intravenous injections of 2 0—2.5 g of AMD It is possible that acute administration of AMD may exert hemodynamic influences other than those of chronic oral medication

In the series where the cardiac output was determined simultaneously with the renal clearances, it decreased in four of six patients with small change in peripheral resistance. The marked increase in renal fraction of cardiac output in these cases points to a special renal effect under the circumstances of this study

Bradycardia of moderate degree during treatment with AMD has been previously described (3 9) There was no such

tendency at rest in the present series. During exercise, however a moderate reduction in heart rate was observed, resulting in an elevated stroke volume.

Of special interest is that the reduction of arterial blood pressure at rest not only persisted during exercise but was even still more marked without any unfavorable effect on cardiac performance. Thus several hypertensive patients showed a quite normal blood pressure reaction during exercise under treatment with AMD There was no tendency to post exercise hypotension. It is therefore unlikely that the hypotensive effect of AMD is mediated by a depressive action of AMD on myocardium.

The lack of any disadvantageous effect of AMD on glomerular filtration rate or renal plasma flow agrees with the findings of Cannon et al. (5) and Onesti et al. (18) Nor has any deterioration of renal function been observed during long term treatment.

In the present series AMD was as effective in primary as in secondary hypertension. In patients with hypertension grade III—IV and high initial blood pressure it was often reduced. Cannon et al. (5) found several cases of severe and malignant hypertension to be sensitive to the drug On the other hand they found sympathectomized patients to be resistant to treatment with AMD which has, however not been apparent in the present series.

Treatment with AMD has in the present series proved to be especially effective in labile, easily excitable and restless patients with high, fluctuating blood pressures. The moderate sedative action might be of special value in these patients.

AMD differs favorably from the ganglionic and sympathetic blocking agents in that there is an appreciable blood pressure reduction not only in the standing position but also when the subjects are recumbent (3 5 7 9 11 15 21) However the blood pressure decrease is more pronounced in the erect position. Over dosage will provoke orthostatic reactions with its risks in elderly persons with rigid vessels. With adequate dosage it has been possible to avoid orthostatic reactions in most cases.

The decrease in systolic pressure is greater than that of the diastolic (5 15 18 21)

The main drawback of AMD has been the development of tolerance, necessitating successively increased doses. Doses of more than 1.5 g per day have often been accompanied by a persisting tiredness and sleepiness. A combination therapy with other hypotensive agents has therefore become standard the dose of AMD not exceeding 1.5—2.0 g per day This has also been advocated by others (1 21)

An initial dose of 0.25 g 2—3 times per day has been found to be suitable in most cases. During hospital stay the dose may if necessary be increased by 0.25 g per day every second to third day under continuous control of the blood pressure in both the lying and standing positions. The interval between dose increments during ambulatory treatment should be one week or more. If an adequate blood pressure response is not achieved by a daily dose of 1.0—1.5 g AMD a saluretic agent in a fixed dose might be added, and if necessary also other hypotensive agents. In many cases it might be advisable to start the therapy with a combination of AMD and a saluretic agent.

Apart from sedation and orthostatic reactions AMD has been found to produce relatively few side-effects. Gillespie (10) reported two cases of febrile reactions in

Table Y Side effects of a methyldopa (mild side effects + moderate ++ and severe +++) Figures represent number of subjects

	+	++	+++
Sedation	2	6	4
Orthostatic troubles, dizziness	2	3	2
Cerebral deterioration (in combination with orthostatic reactions)	—	1	—
Obstipation	—	1	—
Impotence	—	—	1
Failure of ejaculation	—	1	—

served in some cases, as also reported by others (1 2 3 9) No alterations in heart rhythm or rate attributable to AMD have been observed

No febrile reactions or clinical signs of toxic influences on liver or blood have been observed After more than five months of treatment a slight to moderate increase of the eosinophilic white cells was found in three patients four patients showed a moderate relative lymphocytosis The significance of these findings remains obscure. Liver tests including GOT and GPT did not show any alterations that might be due to the treatment with AMD Renal function was unchanged

Discussion

It might be concluded from the results in the present series and from other reports (1 3 5 7 9 11 15 18 21 25) that alpha methyldopa (AMD) significantly reduces blood pressure in the hypertensive man. The exact mechanism is still not clarified

Initially it was assumed that the main factor would be an inhibition of norepinephrine synthesis. It was however observed in animals that there was no parallelism between inhibition of the decarboxylation process and lowering of the blood pressure, the latter appearing after a latent period of several hours (12) The excretion of norepinephrine or its metabolites in the urine is unaltered during AMD treatment, indicating that the inhibition of decarboxylation is not the primary hypotensive mechanism (3)

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combination with pathological liver tests with the racemic form Documented cases of liver damage or allergic reactions with the l isomer have not yet been published Except for one case of reversible agranulocytosis (13) no hematologic changes have been reported. Until further experiences of long term treatment have been gained, it is advisable however to watch for such reactions. Reactive depression has been observed with the racemic form (11) but has not been reported to occur with the l isomer

Summary

1 Alpha methyldopa (AMD) — *a* methyl 3 4-dihydroxy DL-phenylalanine — is in the experimental animal a potent inhibitor of i.a. the decarboxylation of dopa to dopamine, leading to blocking of norepinephrine synthesis.

2. In man the l isomer of alpha methyldopa significantly reduces blood pressure this does not seem to be due to a decreased formation of norepinephrine. An influence on norepinephrine storage in the tissues instead might be a possible mechanism.

3 Alpha methyldopa seems to lower blood pressure through a decrease in total systemic resistance, as cardiac output is unchanged It has not been shown to have any unfavorable influence on glomerular filtration rate or renal plasma flow

4 Alpha methyldopa is effective in lowering blood pressure in about 3/4 of all cases in all forms of hypertension The blood pressure is reduced in both the lying and the standing positions, but orthostatic reactions appear sometimes.

5 During long term treatment tolerance may develop which requires successively increased doses to maintain the hypotensive effect.

6 Persisting sedation is a common side effect, when doses of more than 1.5—2.0 g per day are used

7 To avoid sedative side effects it is proposed to use alpha methyldopa in doses not exceeding 1 0—1.5 per g day and in combination with a saluretic drug.

Acknowledgements

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A Comparative Investigation into the Controllability of three Oral Anticoagulants (Cumethoxethan, *Bishydroxycoumarin* and Acenocoumarol)

By

H. Ch. Hart and L. van Wijk

Since the therapeutic introduction of oral, indirectly active anticoagulants with an anti-vitamin K effect, the choice of the anticoagulant has been determined by a number of factors, namely the physician s preference for a so-called long-acting or a short-acting drug his experience with a given drug — which among other things is dependent on the time of its commercial introduction his opinion regarding the controllability of the drug the incidence of escapes and complications, i. e. in general the way in which a satisfactory and safe hypocoagulability can be maintained and finally the influence exerted by the publicity systems of the pharmaceutical industries.

In the Utrecht thrombosis service, the impression was gained that the anticoagulant Cumethoxethan (1 methoxy-2,2-bis-(4-hydroxycoumarinyl 3) ethane = Dicumoxane A. C. F) is less easily controlled i. e. causes more frequent escapes than the other two widely used anticoagulants Bishydroxycoumarin (3,3'-methylene-bis-(4-hydroxycoumarin) = Dicumol A.C.F) and Acenocoumarol (3/alpha-acetonyl-4-nitrobenzyl/-4-hydroxycoumarin = Sintrom Geigy)

In view of this it was considered desirable to institute a comparative investigation into a number of factors which determine the controllability of an anticoagulant in our thrombosis service, in which the vast majority of patients are followed up as out-patients.

Methods

Organization of the Utrecht Thrombosis Service (2)

The indications for treatment and the choice of the anticoagulant are determined, not by the thrombosis service but by the patient family doctor or specialist, who requests the assistance of the thrombosis service for follow-up and advice as to dosage.

Nurses from the out patient clinic obtain 2 ml blood in 3.8 % sodium citrate (ratio 1 9) collected in non-siliconized material. After storage of the blood sample at room temperature for maximum of 4—5 hours, the

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Table I Three groups of 405 patients each, not selected as to age year of institution of treatment sex and long-term or short-term treatment (for orientation only statistically unvalued)

	Cumethoxethan	Bishydroxy coumarin	Aceno-coumarol
Total no. of therapeutic months	4,372.01	5 443.37	3,567 79
Total no. of escapes	371	985	433
Total no. of haemorrhagic complications	54	110	28
Total no. of thrombo-embolic complications	14	17	9
Total no. of therapeutic months *divided by* total no. of escapes	11 78	5.25	8.26
Total no. of therapeutic months *divided by* total no. of haemorrhagic complications	80.9	49 4	127.7
Total no. of therapeutic months *divided by* total no. of thrombo-embolic complications	312.2	320.1	397.5

prothrombin time is determined by Quick s one-stage method, using the thromboplastin Thrombokinase-Ca "Geigy The results of determination are recorded in seconds, and mention is also made of the prothrombin time determined on the same day in an untreated control. The prothrombin times of patient and control are recorded in a graph, which also indicates the anticoagulant used its dosage, the follow-up dates, the patient s age, the indication(s) for treatment, and such haemorrhagic and/or thrombo-embolic complications as may have occurred during medication. The dosage and the date of the next follow-up are established by the physician of the thrombosis service and the patient or his family is informed of his advice, usually by telephone The patient s family doctor or specialist is informed by a written message.

The object of the physician of the thrombosis service is to attain a prothrombin time 1 1/2 to 2 1/2 times as long as the control time which control time may vary between 11 and 14 seconds. To give an impression of the extent of the activities of the Utrecht thrombosis service in 1960 a total of 38,887 prothrombin time determinations were carried out in 2,353 patients.

FACTORS INFLUENCING CONTROLLABILITY

We regard the following criteria as important in assessing the *practical* controllability of an anticoagulant in a *routine* thrombosis service.

1 *The number of escapes observed.* As escape we regard in this paper

a) A prothrombin time 3 times as long as the control time and longer

b) A prothrombin time between 30 seconds and 3 times the control time, when the patient was advised to discontinue medication on the day of determination or the next day (it is assumed that the physician of the thrombosis service regards a prothrombin time between 30 seconds and 3 times the control time as unacceptable, i. e. as an escape when he reduces the dosage to 0 tablets on the day of determination or the next day)

2. *The incidence of haemorrhagic complications.* All haemorrhages, including the insignificant, are recorded.

3 *The incidence of thrombo-embolic complications*

4. *The percentage of prothrombin time determinations which,* during medication *falls within the limits indicating the therapeutic zone* (1 1/2—2 1/2 times the control time)

5 *The average daily number of tablets per patient.* The practical value of an anticoagulant diminishes when the number of tablets (or part of a tablet) given daily is either too large or too small.

Material

Quantitative data obtained

From the patient material of the thrombosis service, we selected three groups of 405 patients each, treated with Cumethoxethan, Bishydroxycoumarin and Acenocoumarol, respectively The number of escapes, haemor

Table II Three groups of 50 patients each, individually comparable as to age, sex, year of institution of treatment and long-term or short-term treatment (statistically valid)

	Cumethoxethan	Bishydroxycoumarin	Acenocoumarol
Total age in years	2,920	2,898	2,848
Total duration of treatment in months	668.66	674.06	666.20
Total no. of escapes	42	78	80
Total no. of haemorrhagic complications	8	9	7
Total no. of thrombo-embolic complications	1	1	2
Percentage of prothrombin times above the therapeutic zone	2.70	4.01	6.86
Percentage of prothrombin times within the therapeutic zone	59.16	54.64	64.30
Percentage of prothrombin times below the therapeutic zone	38.14	41.33	28.84
Arithmetic mean of no. of tablets per therapeutic day per patient	2.42	1.13	0.84
No. of mg of active principle per tablet	50	50	4
Dose distribution expressed as no. of daily tablets	0–7	0–4	0–4

Table III Example of elaboration of the Friedman test, concerning number of escapes. Patients are individually comparable as to year of institution of treatment, sex, age and duration of anticoagulant therapy

Treatment started in	Sex	Cumethoxethan				Bishydroxycoumarin				Acenocoumarol			
		Age	Duration (months)	No. of escapes	Assigned rank no.	Age	Duration (months)	No. of escapes	Assigned rank no.	Age	Duration (months)	No. of escapes	Assigned rank no.
1957	♀	61	20.10	1	3	63	22.00	5	1	61	19.50	2	2
1957	♀	88	.80	1	2	70	8.50	1	2	67	7.90	1	2
1958	♀	74	27.44	1	3	75	28.00	2	2	73	27.60	7	1
1959	♂	71	21.50	1	2.5	74	23.50	1	2.5	64	22.70		1
1960	♂	53	10.53	1	2	56	9.90	1	2	52	10.00	1	2

In this manner 3 groups of 50 comparable cases were collected.

rhagic and thrombo-embolic complications per patient was established. We also established the ratio between the total duration of treatment in months on the one hand, and on the other hand the total number of escapes, that of haemorrhagic, and that of thrombo-embolic complications for each anticoagulant. Comparison of the three anticoagulants in terms of the figures indicating the interval (in therapeutic months) at which an escape or a complication occurred, reveals unmistakable differences (table I)

In the Cumethoxethan group, the interval between escapes was 11.78 months; in the Bishydroxycoumarin group it was 5.52 months and in the Acenocoumarol group it was 8.26 months (i. e. therapeutic months). Cumethoxethan, therefore, had the lowest frequency of escapes. Comparison in terms of the incidence of haemorrhagic and thrombo-embolic

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Table IV Sums of rank numbers and corresponding probability as to number of escapes and percentage of prothrombin times above within and below the therapeutic zone in the 3 groups each of 50 comparable patients (Friedman test)

	Sum of rank no.			Probability
	Cumethoxethan	Bishydroxycoumarin	Acenocoumarol	
No. of excessive responses	117	94	89	0.001 < P < 0.005
Percentage of prothrombin times above therapeutic zone	117	97.5	85.5	0.001 < P < 0.005
Percentage of prothrombin times within therapeutic zone	93.5	89	117.5	0.005 < P < 0.010
Percentage of prothrombin times below therapeutic zone	89.5	90	120.5	0.001 < P < 0.005

complications was in favour of Acenocoumarol this drug caused a haemorrhagic complication at intervals of 127 7 therapeutic months, and a thrombo-embolic complication once in every 397 5 therapeutic months. The incidence of haemorrhagic and thrombo-embolic complications in Cumethoxethan treatment was 1 haemorrhage per 80.9 months, and 1 thrombo-embolism per 312.2 therapeutic months. Corresponding intervals in Bishydroxycoumarin treatment were 49 4 and 320 1 therapeutic months, respectively

This heterogenous patient material of 3 groups of 405 patients, however warrants no statistically valid conclusions because the differences in year of institution of treatment, age, etc. are too wide.

So as to ensure statistically justified conclusions, three groups of comparable therapeutic cases were formed. In terms of year of institution of treatment, sex, age and duration of medication in months, comparable cases were collected under each anticoagulant heading In this manner 3 groups of 50 comparable cases were collected for th 3 anticoagulants Cumethoxethan, Bishydroxycoumarin and Acenocoumarol.

The total age in years of the 50 patients in the Cumethoxethan group was 2,920 years that in the Bishydroxycoumarin group was 2,898 years, and that in the Acenocoumarol group, 2,848 years. The total duration of medication in months in the three groups, in the above order of sequence, was 668.66, 674 06 and 666.20 months. A comparison is now possible in terms of escapes, haemorrhagic complications and thrombo-embolic complications (table II) The total number of haemorrhages (8, 9 and 7 in the above sequence) and the total number of thrombo-embolic complications (1 1 and 2) was too small to warrant definite conclusions. The total number of escapes was smallest in the Cumethoxethan group (42) the figures in the Bishydroxycoumarin group (78) and the Acenocoumarol group (80) were unmistakably higher The 3 groups of 50 patients were not comparable in terms of diagnosis. It is to be imagined that — in cardiac decompensation as may occur in coronary sclerosis and atrial fibrillation — the effect of anticoagulants shows fluctuations, partly dependent on the degree of hepatic congestion. In view of this, a larger number of excessive responses can be expected in this group of patients.

The Cumethoxethan group included 22 patients with myocardial infarction or atrial fibrillation the Bishydroxycoumarin group included 8, and the Acenocoumarol group 17 patients of this description. Contrary to expectations, there was no correlation between the large number of patients, in the Cumethoxethan group, in whom cardiac decompensation was possible, and a larger number of escapes.

The arithmetic mean of the number of tablets per therapeutic day per patient was 2 42 for the Cumethoxethan group, 1 13 for the Bishydroxycoumarin group, and 0.84 for the Acenocoumarol group (table II)

The percentage of prothrombin times above within and below the therapeutic zone of

1 1/2—2 1/2 times the control time, was also determined for the 3 groups (table II)

The statistical elaboration of the material was carried out with the aid of the Friedman (1) test (method of M ranks) After assignment of rank numbers to each of the three comparable cases, we established the probability of the difference in the frequency of escapes between the 3 anticoagulants being based on coincidence. In assigning the rank numbers, the figure 3 was assigned to the most favourable case (smallest number of escapes,) the figure 1 to the least favourable case (largest number of escapes) and the figure 2 to the intermediate cases, in these corresponding comparable therapeutic cases. In the case of equals, the number of points available was evenly distributed over the 2 or 3 equal cases (table III)

The sum total of rank numbers regarding the number of escapes for Cumethoxethan, Bishydroxycoumarin and Acenocoumarol was 117 94 and 89 respectively (table IV)

The probability of this rank distribution being based on coincidence is so low (P = 0.005—0.001) as to warrant the conclusion that the difference in incidence of escapes is not based on coincidence. The probability at a given rank distribution is calculated with the aid of the equation

$$\chi^2(k-1) = \frac{12(k-1)K}{nk^3-D}$$

in which k = the number of groups = 3

K = the sum of the squares of the differences between the expected and the observed sums total of rank numbers

n = the number of comparable cases = 50

D = correction factor for equals (ties).

Cumethoxethan, therefore, produces significantly fewer escapes than Bishydroxycoumarin and Acenocoumarol under the conditions prevalent in the Utrecht thrombosis service, using Thrombokinase-calcium (Geigy)

In a similar manner after assignment of rank numbers, it was established whether the 3 groups of comparable patients showed statistically significant differences in percentages of prothrombin times above, whithin and below the therapeutic zone (table IV) After determining the percentage of prothrombin times above, within and below the therapeutic range in each patient, statistical elaboration was carried out in a similar manner with the aid of the Friedman test. In each group of 3 comparable cases in the three series of 50 patients, the rank number 3 was assigned each time to the most favourable case (the smallest percentage of prothrombin times above the therapeutic range, the smallest percentage below the therapeutic range and the largest percentage of prothrombin times within the therapeutic range) etc.

Acenocoumarol significantly showed the largest percentage of prothrombin times above the therapeutic zone; Bishydroxycoumarin had a smaller and Cumethoxethan the smallest percentage in this respect.

In the Acenocoumarol group the prothrombin times were most frequently within the therapeutic zone, followed by Cumethoxethan and Bishydroxycoumarin, in that order

Prothrombin times below the therapeutic zone were least frequent in the Acenoucoumarol group, and more frequent in the Cumethoxethan and Bishydroxycoumarin groups.

Discussion

The primary comparison was made between three groups of 405 patients, considering the incidence of excessive responses, thrombo-embolic and haemorrhagic complications during medication with the anticoagulants Cumethoxethan, Bishydroxycoumarin and Acenocoumarol respectively

Although the three groups of 405 patients were insufficiently comparable to warrant statistical elaboration of findings, the general trend of results corresponded with the results, in terms of escapes, obtained in three groups of 50 comparable therapeutic cases, which were used in statistical evaluation.

The Cumethoxethan group showed less frequent escapes (every 11 78 therapeutic months) than the Bishydroxycou-

marin and the Acenocoumarol group (5 25 and 8.26 months respectively)

A striking feature is that the higher frequency of escapes in the Acenocoumarol group as compared with the Cumethoxethan group was not associated with a higher frequency of haemorrhagic complications on the contrary the former group showed one haemorrhagic complication per 127 7 therapeutic months, while the figure for the latter group was 80 9 months (table I) In contrast to this, Bishydroxycoumarin combined a higher frequency of escapes (1 in 5.25 therapeutic months) with a higher haemorrhagic frequency (1 haemorrhagic complication in 49 4 therapeutic months)

Assessment and comparison of the data on the three groups of 405 patients requires considerable prudence. Cases to be compared must originate from the same period of treatment the year of institution of treatment must be taken into account In the course of the years there has been an unmistakable decrease in the number of haemorrhagic complications occurring per calendar year Since only Bishydroxycoumarin was used during the period 1949 through 1952 (101 of the 405 cases in the Bishydroxycoumarin group fell within this period) the comparison may be unfavourable to Bishydroxycoumarin because the incidence of haemorrhages was high precisely during this first period of anticoagulant therapy while the experience with anticoagulant treatment was still limited.

In comparing the number of haemorrhagic complications, the patient's age must be taken into account for the frequency of haemorrhages increases with increasing age (2) It seems necessary finally to distinguish between long term and *short term treatment*. A period of a few weeks is often required before the hypocoagulable condition has been established. It may well be, therefore, that the majority of escapes and prothrombin time determinations outside the therapeutic zone, are recorded in the initial phase of medication.

In view of these facts it was decided to form three groups of 50 patients each, comparable as to years of institution of medication, sex, age and duration of treatment in months. In these three groups of 50 patients the trend visible in the 3 groups of 405 patients in terms of incidence of escapes, was found to be confirmed. Under the conditions prevalent in the Utrecht thrombosis service, and using Thrombokinase-calcium Geigy" the Cumethoxethan group had a considerably (significantly) lower incidence of escapes than the Bishydroxycoumarin and Acenocoumarol groups (P = 0 005 —0 001)

The percentage of prothrombin times above the therapeutic zone was likewise significantly lower in the Cumethoxethan group (P = 0 005—0 001) which means that the use of Cumethoxethan involves a trend to lower prothrombin times. The cause of this less frequent occurrence of increased prothrombin times in Cumethoxethan treated patients is probably *to* be found in the relatively large number of tablets ingested by the patients. The arithmetic mean of the number of tablets of Cumethoxethan per therapeutic day per patient was 2 42 (1 13 for Bishydroxycoumarin and 0 84 for Acenocoumarol) In these out patients there is no real check as to the ingestion of the actual dose prescribed If several tablets have to be taken in the course of the day there is an increased risk that the patient forgets to ingest part of the daily dose. The disagreeable taste of Cumethoxethan

may be a factor of importance in this respect.

In the case of Acenocoumarol, the daily intake of which is generally less than one tablet, there is a tendency towards higher prothrombin times. The number of escapes in this group was significantly larger than in the Cumethoxethan group as was the percentage of prothrombin times above the therapeutic zone. The percentage of prothrombin times below the therapeutic zone was smallest in the Acenocoumarol group (table II)

Although the number of haemorrhagic complications in the three groups of 50 comparable patients is too small to warrant statistically sound conclusions, it is a striking feature that the larger number of escapes in the Acenocoumarol and Bishydroxycoumarin groups does not seem to be associated with a larger number of haemorrhages.

Of the three anticoagulants, Acenocoumarol showed the largest percentage of prothrombin times within the therapeutic zone (64.3 %) (table II) A strikingly small part of the duration of treatment lies at the hypocoagulable level considered desirable. It should be borne in mind, however that these were out-patients, whose actual taking of the doses prescribed was not supervised. The intervals between prothrombin time determinations averaged 3 weeks, which is of course longer than in clinical cases.

The percentage of prothrombin times below the therapeutic zone was considerably larger than that of prothrombin times above the therapeutic zone for these three anticoagulants. In the Utrecht thrombosis service, dosages are determined with prudence lest haemorrhagic complications occur Bishydroxycoumarin was the most unfavourable in this comparative study It showed a large number of escapes and the smallest percentage of prothrombin times within the therapeutic zone (54 64 %) (table II) As to an explanation of the unfavourable results with Bishydroxycoumarin, both the duration of action of the anticoagulant and the thromboplastin used must be taken into account.

Cumethoxethan is a short-acting indirect anticoagulant Acenocoumarol is also short-acting, but its effect is less short than that of Cumethoxethan. Bishydroxycoumarin is the longest-acting of the three anticoagulants.

The very short duration of action of Cumethoxethan reduces the possibility of cumulation, and therefore the risk of unexpectedly high prothrombin time values. A short-acting anticoagulant shows more marked variations in its anti-vitamin K effect and therefore more marked variations in the concentration of the clotting factor with a very short half-value time (T 1/2) e. g proconvertin (factor VII) than a cumulative long-acting anticoagulant.

However the thromboplastin used (Thrombokinase-Ca "Geigy") must also be taken into account. This thromboplastin has a low factor VII sensitivity In the case of a factor VII insensitive thromboplastin, fluctuations in the prothrombin time due to variations in the factor VII concentration are less apparent in the prothrombin time curve.

When the action of short-acting, low cumulative anticoagulants such as Cumethoxethan and Acenocoumarol, leading to highly variable proconvertin concentrations, is verified with a thromboplastin of low factor VII sensitivity these marked fluctuations in factor VII concentration produce only mild variations in the prothrombin time curve, and consequently lead to more even dosages. When a short

acting anticoagulant is used a factor VII insensitive thromboplastin would seem to be required.

On the other hand the control of long acting cumulative anticoagulants such as Bishydroxycoumarin and Phenprocoumon (Marcumar) with a thromboplastin of low factor VII sensitivity is bound to lead to disagreeable surprises in the form of unexpectedly low factor VII concentrations and therefore, a risk of haemorrhage. In the case of long acting anticoagulants, the use of a thromboplastin of good factor VII sensitivity is required (3).

Summary

In the patient material of the Utrecht thrombosis service a comparative investigation was made into a number of factors of significance in the control ability of indirectly acting anticoagulants. Three groups of 50 comparable cases were selected which were treated with the anticoagulants Cumethoxethan, Bishydroxycoumarin or Acenocoumarol. A comparative study was made of the number of escapes, the incidence of haemorrhagic and thrombo-embolic complications and the percentage of prothrombin times above, within and below the therapeutic zone. In the therapeutic results obtained with the 3 anticoagulants, small but consistent differences were found. Cumethoxethan showed a tendency towards lower prothrombin time values, associated with a small number of escapes. Bishydroxycoumarin showed a large percentage of prothrombin times above and below the therapeutic zone, and a large number of escapes. Of the 3 anticoagulants, Acenocoumarol had the largest percentage of prothrombin times within the therapeutic zone and a trend to higher prothrombin times. A correlation is suggested between these findings, and the anti vitamin K effect per tablet and the duration of action of the anticoagulants considered. In the case of the long-acting Bishydroxycoumarin the thromboplastin used must be taken into account which, because of its low factor VII sensitivity is rather unsuitable for this anticoagulant.

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Congenital Renal Disease, Deafness and Myopia in one Family

By

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In 1927 Alport (1) for the first time described a combination of hereditary renal disease and nerve deafness. This syndrome has since been reported in a number of families in different countries (2, 3 5 6, 8—24) In several instances, the syndrome also included ocular anomalies. Hereditary kidney disorder of this type has often been termed Alport's syndrome in the literature, a designation which will be used in the following. The mode of inheritance has not been analysed conclusively However in the major familial series on record there is much to suggest a partially sex-linked dominant trait — at least as regards the kidney condition (16) Alternatively an autosomal dominant trait has been postulated, more severe in males (7)

Renal disorder

Two different main types of kidney disease have been described in the syndrome. It is chiefly one or other of these two forms that affects a family It seems unlikely then, that one form is homozygous and the other heterozygous.

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In the majority of cases the kidney disorder has exhibited fairly similar features, consisting in sporadic or constant haematuria of early onset and progressive kidney damage. The disease has been more severe in affected men, most of whom have succumbed before the age of 30 years. In women the disorder has shown the same characteristics, but the kidney disease has usually run a milder course. Exacerbations during pregnancy and infections are common. In families affected with the syndrome, albuminuria and haematuria have been demonstrated in infants only a few weeks old In this group the clinical features most closely resemble those of chronic glomerular nephritis. The patients show a very high incidence of nerve deafness and ocular defects.

In a minority of cases the kidney disorder may also be less severe in men. The onset is usually at a later age, often after puberty Deafness is less constant. In these patients, the signs and symptoms most closely resemble those of chronic pyelonephritis. Some authors (15) main-

tain that this type should be assigned to a special syndrome while others express the view that the primary disease is the same (6) The existence of overlapping is suggestive of the latter alternative For some reason persons in this second group are more highly susceptible to renal infection and suffer reiterated episodes of pyelonephritis which finally dominate the clinical picture. Patients examined with electrophoresis have not shown signs of hypogammaglobulinaemia (21) Nor has any general susceptibility to infection been found. As in the first type of kidney disease the men in the pyelonephritis group are also affected earlier and the prognosis is poorer in them than in women

The histopathologic features of the renal disease have been variable in both autopsy and kidney biopsy cases. In the form with early onset the changes have been chiefly of the type associated with chronic glomerular nephritis, with kidney shrinkage. In the later form the pyelonephritic changes predominate. In young persons with a short history of disease, biopsy has as a rule revealed only uncharacteristic lesions. Lökken (10) has described cases of renal dysplasia in siblings with pyelonephritis who succumbed to uraemia at an early age. Dysplastic kidneys have been found to be especially susceptible to infection and Lökken advanced the view that the dysplasia might be the primary lesion of hereditary pyelonephritis Another change maintained by some pathologists to be specific for hereditary nephropathy is the presence of so-called foam cells. However Whalen et al. (24) have demonstrated these cells in a number of other disorders — although there is some predominance of the hereditary kidney diseases.

It may be said then, that no histologic changes in the kidneys pathognomonic of the syndrome have been demonstrated. Biochemical changes seem the more likely explanation There is much to suggest that the primary factor is an enzymatic defect of genetic derivation, which leads secondarily to lesion of the ears, eyes, and kidneys. In three cases of Alport's syndrome in one family Wallace (25) found general amino-aciduria which might bear out this hypothesis. The amino-aciduria may of course, be either primary or secondary to the kidney damage.

In addition to individuals with severe renal disorder both main groups of hereditary nephropathy include families with some members who have signs of only mild kidney disorder Women with slight albuminuria alone are particularly commonly reported (24) A likely inference is that the mild renal derangements occur in heterozygotes while the more advanced forms involve homozygotes.

Ear lesions

Deafness is not an invariable part of Alport's syndrome. However many patients have moderately defective hearing or unilateral deafness which is demonstrable only audiometrically The affected families may include members with hearing defects in whom there are no signs of renal disease. Conversely there may be members with kidney disorder in whom the audiogram is normal. In some families with hereditary renal disease, no ear disorder whatever has been demonstrable although the kidney derangement is of the same type as in Alport's syndrome. There has, therefore, been some discussion as to whether the hearing defect may be different in mode of inheritance from the renal disease (15 16)

The loss of hearing is usually demonstrable at an early age and gradually progresses. Sohar (21) claimed to be able to date the onset of the hearing defect to around the age of 10 years in his series. As in the case of the kidney disorder men are more frequently affected than women and the impairment in hearing is usually more severe in them. Histopathological examination at autopsy of patients with the syndrome has in no instance detected any distinct changes that might account for the loss of hearing. However the clinical features — above all the occurrence of positive recruitment — suggest that the hearing defect is attributable to involvement of the organ of Corti (21). In some publications (22) it has been stated that this group of patients is particularly prone to otitis. Possibly there is in the ear a focus of lowered resistance analogous with that found in the kidneys. Nystagmus and other signs of vestibular damage are not recorded in the literature on Alport's syndrome.

Ocular manifestations

Eye lesions in Alport's syndrome have been reported chiefly from Israel (21). The chief change recorded is cataract — five cases in four families. In two instances spherophakia with secondary myopia, in two only myopia of moderate degree, −3 to −6 diopters, and without changes of the fundi. Several other eye anomalies have been described such as lenticonus, retinal plasia, green pigmentation of the cornea, ocular nystagmus, and retinal detachment (3, 6, 11, 18).

The present paper reports on a family with a similar syndrome. Among seven siblings, three brothers exhibited eye, ear and kidney lesions. The features of the condition differed from those recorded

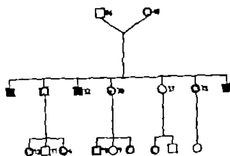


Fig. 1. Family tree.

□ male

○ female.

□ ○ renal symptoms in the form of albuminuria and/or haematuria.

■ fully developed syndrome with renal symptoms, deafness, and myopia.

in the literature in the nature of the eye disorder (severe myopia) and in the course of the kidney disease. In addition, several other members of the family suffered from renal disorder without eye or ear defects.

The three brothers with the full syndrome are reported individually below; these case reports are followed by a brief account of the other members of the family.

Case reports

Case 1 (II 1 in the family tree). Male, 37 years.

Severely impaired vision since early childhood, esotropia, and increasing loss of hearing. Albuminuria constantly present since the initial medical examination at school. No symptoms of pyelonephritis, scarlet fever or measles. During the years 1936 to 1943 he had three bouts of unilateral or bilateral otitis with complications. Like two of his brothers, he had used a hearing aid since he was in his teens. The visual defect remained essentially unchanged after the initial examination in 1932, around 2/60 bilaterally with spectacles. Myopia of −20 diopters and advanced myopic fundus. Convergent strabismus of approximately 25 degrees throughout. Opacities of

the crystalline body and lens developed after 1952.

On examination at the Outpatient Department of Karlskoga Hospital in Nov 1961 his general condition was good, and the lungs and heart showed nothing abnormal on physical examination. Blood pressure 140/90 mm Hg Myopia of —20 diopters, advanced myopic fundus. Visual acuity 2/60 bilaterally The tympanic membranes were somewhat scarred but otherwise normal. An audiogram revealed a purely perceptive loss of hearing of 60 to 70 decibels, the reduction being quite even throughout the speech range. Normal vestibular function. Albumin was present in the urine, 0 4 g/24 hours (Esbach method) The sediment and serum creatinine were normal.

Case 2 (II 3 in the family tree) Male, 32 years.

Earlier records from the period 1932 to 1948 reported severe myopia since early childhood, remaining essentially unchanged at around —22 diopters. Visual acuity approximately 0 1 on the right and somewhat less on the left side with spectacles. An appreciable hearing defect was present already at the age of 8 years this progressed gradually partly in connexion with infections of the upper respiratory tract. From at least the age of 2 years albuminuria was noted, the values ranging from 0.2 to 2 0 g/24 hours. The sediment sporadically showed sparse microscopic haematuria. No symptoms of pyelonephritis, scarlet fever or measles. He was admitted to Karlskoga Hospital for investigation in Sept. 1961 His intellectual level was somewhat low General condition good. Heart and lungs normal on physical and roentgen examination. Blood pressure 130/80 mm Hg ECG normal. Haemoglobin, red and white cell counts, and differential count normal. Liver tests (thymol, alkaline phosphatases, bilirubin in serum, and serum transaminases) were normal. The serum cholesterol according to Bloor was 205 mg/100 ml. Paper electrophoresis with Laurell's method showed normal values for all th serum protein fractions. The turbidity of fasting serum was normal. The eyes showed myopia of —22 diopters on the right and —28 diopters on the left side. Advanced myopic fundus bilaterally Visual acuity with spectacles 0.1 on each side. An audiogram revealed a marked hearing defect of wholly perceptive type. No nystagmus. Roentgen examination of the ears was normal. The 24-hour urinary excretion of protein was 0.2 to 1 0 g (Esbach) The urine was concentrated to a maximal specific gravity of 1 017 — an impairment in the concentration ability of the kidneys. Repeated examination of the urinary sediment including quantitative analysis according to Addis was normal. The serum urea nitrogen and creatinine were normal. The carbon dioxide level and serum electrolytes (Na, K) were normal. Intravenous pyelography was normal. Kidney biopsy was refused by the patient. The 24-hour excretion of amino acids in the urine showed on chromatography elevated values for alanine (86 mg) glutamic acid (228 mg), histidine (245 mg) and a somewhat high value for threonine (59 mg) (Dr G. Hammarsten, Södersjukhuset, Stockholm)

Tests at the Embryologic Institution, Lund culture of white blood cells from peripheral blood was successful and showed normal karotypes with 46 chromosomes, including the sex chromosomes XY. No demonstrable aberrations (Kållén)

Case 3 (II 7 in the family tree) Male, 21 years.

Since earliest childhood greatly defective vision, with acuity around 0.2 bilaterally and myopia of approximately —20 diopters. In 1953 1954 and 1955 retinal detachments involving both eyes for which he underwent several operations. Like two of his older brothers, he had since at least before school age had defective hearing Urinary examination always showed albuminuria from traces to 0.2 g/24 hours. On repeated occasions a few red cells in the urinary sediment. No symptoms of pyelonephritis, scarlet fever measles, or otitis.

Admitted to Karlskoga Hospital in Sept. 1961 His general condition was good. Normally developed mentally Heart and lungs normal on physical and roentgen examination. Blood pressure 120/70 mm Hg Electrocardiogram normal, haemoglobin level, red and white cell counts, and differential count normal. Liver tests normal. Serum cholesterol 170 mg/100 ml. Turbidity of fasting serum normal. Paper electrophoresis according to Laurell showed normal values for all the serum protein fractions. Eye examination revealed myo-

pia of — 18 *diopters* on each side. Numerous flame defects on both fundi. Many floating opacities in the left crystalline body. Minor central lens opacities. Visual acuity 0.1 on the right, finger counting at 1 metre on the left. Ear examination disclosed loss of hearing of about 50 decibels, found audiometrically to be purely perceptive in type. No nystagmus. Urine analysis showed constant albuminuria ranging from 0.04 to 0.1 g/24 hours. The urine was concentrated to a maximal specific gravity of 1.032. Repeated examinations of the urinary sediment including quantitative analysis according to Addis were normal. The serum urea nitrogen and creatinine were normal. The carbon dioxide level and serum electrolytes (Na, K) were normal. Intravenous pyelography revealed nothing abnormal. Kidney biopsy was refused by the patient. The 24-hour excretion of amino acids in the urine showed elevated values for alanine (73 mg), glutamic acid (149 mg), glycine (317 mg), histidine (435 mg) and threonine (151 mg) (Hansmarsten).

Tests at the Embryologic Institution of Lund: culture of white blood cells from peripheral blood successful, showing normal karyotypes with 46 chromosomes including the sex chromosomes XY. No demonstrable aberrations (Källén).

Other members of the family

No known consanguinity in the family. Careful written and oral history-taking revealed no evidence of eye, ear or renal disease in the older generations on either the maternal or paternal side. The father of the three brothers (56 years) had moderate presbyopia and presbycusis, apparently due to age. Otherwise his eyes, kidneys, and blood pressure were normal. The mother (48 years) had no recorded kidney disorder or albuminuria up to 1960. After that date she had several episodes of pyelonephritis symptoms. She was admitted for an exacerbation to the Department of Internal Medicine of Karlskoga Hospital, when the family history was discovered.

On physical examination, the mother' general condition was good. Heart and lungs normal on physical and roentgen examination. Blood pressure 160/100 mm Hg. Albuminuria and moderate amounts of red and white cells and bacteria in the urine. The ears and eyes showed conditions wholly normal to her age.

Of the four siblings of the probands, the brother (II 2, 36 years) had microscopic haematuria, one sister (II 6, 25 years) had had albuminuria since the age of 8 years and at least one lengthy period of haematuria. On examination of this sister the albumin values were between 0.2 and 1.5 g/24 hours (Esbach). Another sister (II: 4, 30 years) had had lengthy periods of albuminuria and oedema of her legs during her three pregnancies. Urinary examination in 1962 showed no albuminuria but three to four red cells in the urinary sediment. All four siblings showed otherwise normal conditions on examination, so that the presence of the full syndrome can be ruled out.

Finally of the siblings' nine children five showed signs of renal disease in the form of albuminuria or traces of albuminuria (Esbach). In one of these cases the albuminuria may have been due to recurrent pyelonephritis. The conditions (audiogram included) were otherwise normal in all of them, and neither hearing nor vision was defective.

Discussion

To summarize the family history: Among seven siblings three brothers showed the same combination of congenital anomalies consisting in severe myopia, progressive nerve deafness, and slight albuminuria. One of the brothers had microscopic haematuria periodically. In another kidney function was impaired in that the capacity for concentration was reduced. None of them as yet show signs of severe kidney damage. The urea nitrogen and serum creatinine were normal in all three brothers. Two of them had had frequent attacks of otitis as children. None of them have had scarlet fever or measles, and their histories record no mention of any infection common to them all. Paper electrophoresis gave normal results in the two brothers so examined, so there is no evidence of hypo-

gammaglobulinaemia The hearing defect developed before school age and was of purely perceptive type The myopia was severe with values down to — 20 to — 30 diopters. All three brothers had changes of the ocular fundi and impaired visual acuity and one of them had had repeated retinal detachments. The excretion of amino acids in the urine was determined in two of the brothers and abnormally high values were recorded for some amino acids. The pattern was not uniform in the two cases, but a feature common to both was an increased secretion of alanine glutamic acid histidine and threonine

This combination of congenital anomalies was not demonstrable in the rest of the family But several members had renal disorder The mother had for 12 months had sporadic albuminuria attacks of pyelonephritis and abnormal sediment The fourth brother had sparse microscopic haematuria Of the three sisters one had had sporadic albuminuria and haematuria since the age of 8 years and another had had lengthy periods of albuminuria during her three pregnancies. Of the siblings nine children five showed signs of renal disease in the form of albuminuria The siblings and their children were otherwise healthy

None of the other family members exhibited the deafness described in the three brothers, nor myopia. No consanguinity is known The auditory and visual defects in the three brothers were so striking that any further instance should have been noticed On the other hand histories can never rule out isolated albuminuria or haematuria of the type recorded In the older generations, who were not examined audiometrically the possible presence of slight nerve deafness not noticed by the person in question cannot be dismissed

To the best of my knowledge the literature contains no cases wholly analogous with those reported in the present paper

The coexistence of congenital anomalies of the kidneys and ears, and in some instances also of the eyes, is familiar and has been described in some twenty families in a number of different countries. The cases published earlier have all exhibited fairly similar organic manifestations and the whole symptom complex has often been designated collectively as Alport s syndrome

The cases reported in the present paper show features partly corresponding with and partly differing from Alport s syndrome.

As in most instances of Alport s syndrome, it was only the men who exhibited the full combination of lesions, while the women suspected of being affected showed only signs of mild renal disease.

The kidney disorder differed in several respects from that described in the literature

1 The earlier cases have been characterized chiefly by constant or periodic haematuria while in the present family albuminuria predominated and haematuria occurred only sporadically

2 Although two of the three brothers were over the age of 30 years and the onset of the renal disease had been early only one of them showed signs of impaired kidney function — consisting solely in a reduced power of concentration while the serum urea nitrogen and creatinine levels showed normal values. In earlier cases, the greater number of affected men with early renal symptoms succumbed before the age of 30 years. In the remaining group of men who survived for longer periods, the onset was later and the clinical features cor

responded with pyelonephritis — which was not true in the present case.

The perceptive deafness exhibited the same characteristics as in cases described earlier nerve deafness appearing before or at school age and gradually progressing As in two of the three brothers reported in this paper reiterated attacks of otitis are recorded in several instances in the literature.

The ocular lesion in the present cases consisted in severe myopia with myopic fundus and considerably reduced visual acuity One of the brothers also had strabismus. Severe myopia has not been described earlier in Alport's syndrome, only moderate myopia in four cases which was not accompanied by changes of the fundi (21) Retinal detachment has been recorded in one case earlier but this was not combined with severe myopia. The changes most commonly associated with Alport's syndrome have been spherophakia and cataract, neither of which were present in the cases described in this report.

A deranged excretion of amino acids was noted while the patients were on a normal diet, but the import of this observation remains to be determined.

The family reported in the present paper showed combination of kidney ear and eye lesions not wholly corresponding to those described in the literature in cases of hereditary disorder of those organs. The familial occurrence of diseases involving either the kidneys or eyes or ears is recorded in a fairly large number of instances. But involvement of all three organs has hitherto been observed only in cases designated as Alport's syndrome. This raises the question as to whether the symptom complex described in the present paper is not a variant of Alport's syndrome.

Summary

A family is described in which three brothers had a combination of congenital anomalies consisting in severe myopia progressive nerve deafness, and slight albuminuria. Several other members of the family had sporadic albuminuria and haematuria. The disease affects the same organs as the condition known as Alport's syndrome, from which it differs in the nature of the ocular disorder (severe myopia) and in the milder course of the renal disease — manifested chiefly as albuminuria and in lesser measure as haematuria.

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Polycythaemia in Anoxic Patients with Circulatory Failure

By

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The role of anoxia in the regulation of erythropoiesis is generally recognized. Inhabitants of high altitudes are known to have elevated haemoglobin and red cell levels and artificial lowering of the oxygen tension in the inspired air also leads to an increase in the oxygen carrying capacity of the blood so that normal oxygen content of arterial blood is maintained until very low oxygen tension values are reached.

Compensatory polycythaemia is not unknown to the clinician. Polycythaemia is a common feature in congenital heart diseases, and patients with respiratory insufficiency usually, though not always, have elevated red cell counts. However, the compensatory polycythaemia in advanced respiratory cases is often less pronounced than could be expected on the basis of arterial desaturation, and in congestive heart failure polycythaemia is quite rare. This discrepancy between the stimulus (anoxia) and response (polycythaemia) has been noted by several authors (9, 11, 12, 15) and various explanations have been suggested. The most widely accepted theory presumes that infection, which is often an important factor in respiratory anoxia, leads to iron deficiency and/or depression of erythropoiesis. Lowered serum iron values in patients suffering from anoxia of different types are reported by Wilson et al. (15), Hedlund (6) and Marx (9). Lowered mean corpuscular haemoglobin concentration are also common findings in anoxic subjects (4, 6, 9, 13, 15). According to Shaw and Simpson (11) patients with anoxia of long standing show signs of iron deficiency (normal or high plasma iron binding capacity, high plasma iron clearance) but not those of infection.

The possible effect of circulatory factors on the compensatory polycythaemia has yet gained but little attention. The aim of the present study is to evaluate the effect of insufficient cardiac output on the anoxic polycythaemia, and for this purpose some haematological, oxymetrical and haemodynamic data were analyzed in a group of patients with congenital and acquired valvular heart disease.

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Table I Haematological data of subjects with arterial anoxaemia

Diagnosis	Arterial O_2 satur	Mixed ven. O_2 satur	Hb (g/l)	Erythrocytes (mill/mm³)	Packed cell volume	MCH (pg)	MCHC (%)	MCV (mμ³)	Leukocytes (1,000/mm³)
A. Venous oxygen saturation within normal limits n = 10									
Eisenmenger syndrome	92.0	76.0	195	6.65	67	29.4	29.2	101	5.70
Fallot's tetralogy	82.5	75.1	192	7.22	74	26.6	26.0	103	6.30
Fallot's tetralogy	93.0	75.0	220	6.73	78	32.6	28.2	116	5.00
Eisenmenger syndrome	92.5	74.0	168	6.56	49	25.6	34.2	75	6.60
Eisenmenger syndrome	83.5	69.5	210	7.25	72	29.0	29.2	99	6.40
Patent ductus arteriosus	81.5	68.3	206	9.00	69	22.9	29.8	77	6.50
Ventricular septal defect	*83.0*	*66.5*	*169*	*5.78*	*62*	*29.2*	*27.3*	*107*	8.40
Fallot's tetralogy	92.0	66.5	164	5.66	45	29.0	36.4	79	7.20
Eisenmenger syndrome	92.0	66.0	133	4.72	51	28.2	26.0	108	12.00
Mitral stenosis	94.0	66.0	124	4.97	47	25.0	26.4	95	7.00
Mean	88.6 ±1.8	70.3 ±1.4	178 ±10	6.45 ±0.35	61 ±4	27.7 ±0.8	29.3 ±1.0	96 ±4	6.98 ±0.44
B Venous oxygen saturation lowered. n = 25									
Fallot's tetralogy	78.8	65.0	167	7.20	62	28.4	28.4	86	
Mitral stenosis	91.2	65.0	107	4.00	39	23.7	27.4	97	
Atrial septal defect	88.8	65.0	138	5.94	51	23.2	27.0	86	6.30
Pulmonary arteriovenous fistula	83.0	64.0	150	5.47	55	27.4	27.2	100	6.70
Pulmonary artery hypoplasia	92.0	63.5	115	4.34	45	26.5	25.5	104	6.50
Patent ductus arteriosus	85.0	63.0	165	6.10	52	27.0	31.7	85	
Ventricular septal defect	93.3	62.0	110	4.10	40	17.5	22.0	79	7.40
Ventricular septal defect	89.0	62.0	151	6.08	53	24.8	28.5	87	8.80
Patent ductus arteriosus	83.7	61.2	128	4.50		28.4			
Ventricular septal defect	83.0	59.0	110	6.30	45	17.5	24.4	71	12.60
Mitral stenosis	93.4	59.0	118	3.60		30.8			
Atrial septal defect	87.4	58.3	125	4.42	42	28.3	29.8	95	3.70
Mitral insufficiency	94.0	57.5	124	4.78	47	25.5	26.4	98	10.90
Ventricular septal defect	*87.0*	*57.5*	*150*	*3.82*	*48*	*25.8*	*31.3*	83	8.80
Mitral stenosis	92.3	55.0	120	4.58	43	26.2	27.9	94	4.00
Patent ductus arteriosus	93.5	54.0	81	4.08	35	19.9	22.8	86	6.80
Ventricular septal defect	82.0	53.1	154	4.95	53	31.1	29.1	107	4.40
Atrial septal defect	86.0	50.0	121	5.54	48	22.[illegible]	25.1	87	6.50
Mitral stenosis	92.5	49.0	115	4.94	44	23.3	26.1	89	5.70
Mitral stenosis	92.5	49.0	128	4.30	43	29.8	29.7	100	8.30
Mitral stenosis	91.4	44.0	110	4.56	40	24.1	27.5	88	7.20
Pulmonary hypertension (Multiple embolism?)	91.0	44.0	117	4.00	44	29.3	26.6	110	7.90
(Eisenmenger?)	90.5	39.5	107	4.52	40	23.5	26.8	88	5.60
(Eisenmenger?)	87.0	39.0	130	5.12	53	25.4	24.5	103	14.20
Mitral stenosis	81.0	21.5	104	4.42	40	23.5	26.0	91	9.50
Mean	88.4 ±1.0	54.0 ±2.2	126 ±4	4.94 ±0.18	46 ±1	25.3 ±0.7	29.0 ±.07	92 ±2	7.59 ±0.45

Material and methods

The material comprised 132 cardiac patients on whom a thorough cardiological examination, including cardiac catheterization, was performed during the period 1957—1961. The age of the patients varied between 14 and 50 years, the sex incidence was 50 males and 82 females. The diagnoses as well as the haematological and oxymetrical data of the anoxic cases are given in table I A and I B. The arterial oxygen saturation was lowered (< 93 %) in 33 patients, and 50 had lowered mixed venous oxygen saturation (≷ 65 %). Only in a few cases were signs of circulatory congestion present.

The heart catheterization was performed with standard technique. The arterial blood samples were drawn either from the brachial or from the femoral artery, the mixed venous samples were usually obtained from the pulmonary artery, in some cases from the right ventricle, right atrium, and/or central systemic veins.

The oxygen saturation and oxygen content of the samples were determined oxymetrically, the oxygen content was also controlled with the van Slyke manometric method; both methods gave consistent results. The oxygen consumption was measured during the catheterization with the catharometric Hartmann-Braun apparatus. Cardiac output was calculated from the Fick formula. The cardiac index is given as cardiac output per square meter body surface. The cardiac output was not determined in all cases, and consequently the insufficient cardiac output is mainly expressed in terms of venous anoxaemia. Standard haematological techniques were used for the determination of the haematological status.

Results

In order to test the correlation between venous anoxaemia and haemoglobin concentration, the haemoglobin values of subjects with normal and lowered mixed venous oxygen saturation were compared. The average value in the normal venous saturation group (82 cases, venous saturation > 65 %) was 124.2 ± 2.6 g/l and in venous anoxaemia group (saturation ≷ 65 %, 50 cases) 128.5 ± 2.8 g/l. This difference is not significant, and can be accounted for by the slightly lower average arterial oxygen saturation in subjects with venous anoxaemia (92.9 ± 0.7 % resp. 96.2 ± 0.3 %). Thus, in the present material, *venous anoxaemia has no distinct correlation with the haemoglobin values.*

The group of 33 subjects with arterial anoxaemia was submitted to more detailed study. In table I A and I B the diagnoses, the arterial and mixed venous oxygen saturation and haematological data of the subjects are given. Table I A comprises the cases with normal, table I B those with lowered mixed venous oxygen saturation. As seen, the arterial oxygen saturation is of the same magnitude in both groups. On the other hand, there is a striking difference between the haematological status in subjects with and without venous anoxaemia: while in the group with normal venous oxygen saturation the haemoglobin, erythrocyte count and packed cell volume are markedly elevated as might be expected on the basis of the arterial desaturation, in combined arterial and venous anoxaemia the red blood values are within the normal range (average haemoglobin in whole material 125.9 ± 1.6 g/l, in arterial and venous anoxaemia 126.0 ± 4.1 g/l, and in the group of arterial, but not venous anoxaemia 178.1 ± 10.1 g/l). In the two groups with arterial anoxaemia, the difference between haemoglobin, erythrocyte count and packed cell volume is statistically significant (bottom lines, table I A and I B).

In fig. 1 the haemoglobin values in the arterial anoxaemia group are plotted against mixed venous oxygen saturation

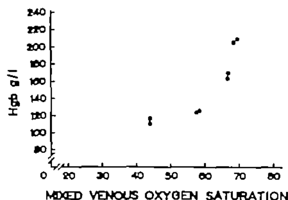


Fig 1 Mixed venous oxygen saturation and haemoglobin content in a group of 35 subjects with arterial anoxaemia (O % < 95)

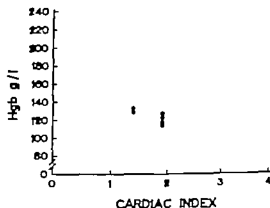


Fig 2. Cardiac index (cardiac output per square meter body surface) and haemoglobin content in a group of 26 subjects with arterial anoxaemia (O % < 95)

The distribution suggests two distinct groups below venous oxygen saturation of 60 % the average haemoglobin runs almost horizontally while at higher venous oxygen saturation the haemoglobin rises steeply showing a good correlation with the venous oxygen saturation The positive correlation is easily explained as if arterial O_2-saturation, cardiac output and oxygen consumption were assumed to be constant, the venous oxygen saturation would be solely dependent on the oxygen capacity (haemoglobin content) of the blood The mean values of haemoglobin and venous oxygen saturation in the subjects with normal venous saturation are in good accordance with this explanation However in the area of low venous oxygen saturation the venous saturation is almost independent of the haemoglobin level and most likely varies with cardiac output, as the individual variations in the oxygen consumption are insignificant. In fig 2 the haemoglobin values in 26 cases of the arterial anoxaemia group are plotted against cardiac index. Again two groups are recognized in cases with normal or moderately lowered cardiac index as well polycythemic as normal or low haemoglobin values are met, whereas in cases with markedly lowered cardiac index the haemoglobin values are either normal or low but never polycythemic. There is also a significant difference between the haemoglobin values in cases with cardiac index above and below 2.0 l/m² (161 7 ± 10 1 g/l resp 122 8 ± 4 3 g/l) *According to the present data it seems that while anoxic subjects with normal cardiac output (normal mixed venous oxygen saturation) usually react to the anoxia with polycythemia anoxic subjects with markedly lowered cardiac output do not.*

As regards the corpuscular contents, the mean corpuscular haemoglobin (MCH) is somewhat lower in subjects with venous anoxaemia, the difference is, however not statistically significant. A similar difference is found also between the mean corpuscular volume (MCV) in both groups, while the mean corpuscular haemoglobin concentration (MCHC) was of the same order in the two groups.

The leukocyte count was elevated in 5 cases with venous anoxaemia but only in one subject with normal venous oxygen

saturation. The average leukocyte count was also slightly higher in venous anoxaemia (table I A and I B, bottom line). Subjects with leukocytosis had slightly lower haemoglobin values than those with normal leukocyte count: in the 5 cases with leukocytosis in the combined arterial and venous anoxaemia group the haemoglobin averaged, 123.6 ± 6.7 g/l, and in the 20 cases without leukocytosis, 126.4 ± 6.7 g/l.

Discussion

According to the present results the compensatory polycythaemia observed in anoxic subjects with normal venous oxygen saturation is not manifested when the venous oxygen saturation is below the normal range, i. e. when the cardiac output becomes insufficient with regard to the oxygen needs of the organism. As to the cause of this, the present data offer but little information. The reason probably should be sought in altered circulatory circumstances which may lead to changes in the perfusion of different tissues including those essential for the maintenance of erythropoiesis. The following possibilities may be considered.

1. *Deficient formation of the erythropoiesis-stimulating substance (erythropoietin)* due to anoxia in its site of origin. This, however, seems unlikely, as tissue anoxia, whether general or local (kidney), is believed to stimulate the formation of erythropoietins (7). The finding of Bonsdorff and Jalavisto (2) that erythropoietins are present in the blood of subjects with respiratory as well as circulatory anoxia, whether the patients were polycythaemic or not, is also in disagreement with this assumption.

2. *Failure of the bone marrow to respond to the stimulus with increased erythropoiesis because of local want of oxygen.* The statement of Rosin and Rachmilewitz (10), Magnussen (8) and Astaldi et al. (1) that hypoxia in vitro (less than 5 athmospheric volume per cent O_2) depresses erythropoiesis in bone marrow transplants is in accord with this, though it may be questionable whether the difference in the bone marrow oxygen tension in pure arterial and combined arterial-venous anoxaemia is great enough to account for a marked difference in erythropoiesis.

3. *Iron deficiency due to infection or poor iron absorption in impaired intestinal circulation.* This could be in accordance with the low serum iron values reported by several authorities (see introduction). The slightly lowered MCH and MCV in combined arterial and venous anoxaemia in the present material might also be interpreted as indicating a moderate iron deficiency. However, the difference in MCH and MCV values between subjects with normal and lowered venous oxygen saturation is not marked enough for interpreting the difference in red blood values in terms of iron deficiency, though this may well be considered as a part factor. The role of infection in the possible iron deficiency, and as a part factor in the lack of polycythaemia in combined arterial and venous anoxaemia in the present material cannot be excluded. It cannot, however, be the only or even the main factor, as most subjects with venous anoxaemia had no leukocytosis and no other signs of infection but still had haemoglobin and erythrocyte values definitely below those in subjects with normal venous oxygen saturation.

4. *Increased red cell destruction might balance increased red cell formation.* This is supported by the statement of Ehrström (5) that anoxic subjects with heart failure show simultaneous signs of increased

erythropoiesis (reticulocytosis) and increased red cell destruction (increased urobilin excretion). Only a minor number of our patients with a low cardiac output had manifest symptoms of circulatory congestion.

5. *A real increase in the erythrocyte mass might be masked by an equal increase in the plasma volume.* Increased plasma volume in certain types of circulatory failure is reported by Harvey (5), Hedlund (6), Zissler (16) and Shaw and Simpson (11). As indicated above, the clinical signs of circulatory congestion were rare in the present material. On the other hand some observations suggest that in cyanotic congenital heart diseases the plasma volume is often decreased and blood volume remains at the normal level (14). Thus the low output group of the present material cannot be considered homogenous in regard of the blood volume, and the lack of polycythaemia in combined arterial and venous anoxaemia can hardly be explained adequately in terms of increased plasma volume.

To analyze the factors responsible for the failure of anoxic patients with low cardiac output to develop polycythaemia a carefully controlled experimental study of circulatory and haematological variables were needed.

Summary

In order to study secondary polycythaemia in anoxic subjects with failing circulation, oxygen saturation of arterial and mixed venous blood and cardiac output were compared with the peripheral blood status in a group of 132 patients with cardiac diseases. Of this material 35 subjects had arterial and 50 subjects venous anoxaemia. The following results were obtained.

1. The venous anoxaemia had no correlation with the haemoglobin values, if the arterial oxygen saturation was not taken into account.

2. In arterial anoxaemia with normal venous oxygen saturation the haemoglobin content, erythrocyte count and packed cell volume were significantly elevated.

3. In subjects with combined arterial and venous anoxaemia the haemoglobin content, erythrocyte count and packed cell volume were only slightly higher than without anoxia and significantly lower than in subjects with arterial anoxaemia of the same degree, but with normal venous oxygen saturation.

4. In subjects with arterial anoxaemia having a low cardiac output the haemoglobin content, erythrocyte count and packed cell volume were significantly lower than in anoxic subjects with normal cardiac output. Compensatory polycythaemia was encountered only in the latter group.

5. In subjects with combined arterial and venous anoxaemia the MCH and MCV values were slightly lower than in subjects with arterial anoxaemia and normal venous oxygen saturation. There was no difference in the MCHC in the two groups.

6. The leukocyte count was slightly higher in subjects with combined arterial and venous anoxaemia than in those with lowered arterial but normal venous oxygen saturation.

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The Beck-I Operation for Angina Pectoris

Medical Aspects

By

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Ischaemic heart disease is one of the most important problems in medicine today In the highly industrialized countries of the western world it is a dominant cause of death. During 1960 more than 5,000 patients were admitted to the medical department of this hospital. In almost one case in twenty the diagnosis was cardiac infarction and one-fifth of these patients died within a month. The incidence of, and primary mortality from cardiac infarction were thus of the same order of magnitude as in other parts of Sweden (4 5 6, 7 10 19 21)

The basic factors in ischaemic heart disease are atherosclerosis and thrombosis. Unfortunately neither unsaturated fatty acids nor anticoagulants can increase the circulation in the ischaemic myocardium. Despite all research, palliation by nitrites remains standard treatment for angina pectoris.

It was not surprising therefore, that the reports published in 1956 and 1957 by Beck (1 2 3) from Cleveland, U.S.A. excited interest and optimism. Beck aim was to intensify by surgical means the formation of collateral channels to the ischaemic region from better nourished areas of the heart. His reasoning briefly was as follows. In coronary insufficiency death can be caused either by purely muscular failure of the ischaemic myocardium or by ventricular fibrillation. Beck believed that the ventricular fibrillation was precipitated by the oxygen differential in the transitional region from anoxaemic to healthy myocardium, which thus acted as a trigger mechanism (13) Cases in which apparent death from cardiac infarction was followed by full recovery in response to external cardiac massage and defibrillation of the ventricles suggest that Beck s theory was essentially correct, although the oxygen gradient may not be alone responsible for the trigger mechanism (15)

That the Beck operation does augment the intercoronary anastomoses was evident from the following observations in dogs.

1 Ligation of the descending branch of the left coronary artery caused death from ventricular fibrillation in 70 per

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Table II Deaths during the postoperative observation period

Age (yrs)	B.P (mm Hg)	Beck op.	Remarks
62	230/120	1958	Malignant arteriosclerosis, albuminuria, left cardiac enlargement. Advised against operation, which was performed at his urgent request. Died 4 days later of fresh infarct.
45	240/150	1958	Malignant arteriosclerosis. Cerebral haemorrhage 1954. Peet operation 1957 Cardiac infarction 1958. Left cardiac enlargement. Greatly improved by Beck operation. Died of gastric haemorrhage in 1960
38	250/140	1958	Malignant arteriosclerosis, lef cardiac enlargement. No relief from Beck operation. Died of cardiac infarction in 19.9.
54	Normal	1959	Progressive angina pectoris for 8 years. Alcoholic. Subjective improvement after Beck operation. Died of cardiac infarction in 1960
63	180/110	1958	Severe angina pectoris for 10 years, blood cholesterol 620 mg/100 ml. Ligation of internal mammary artery 1958, little improvement. Greatly improved by Beck operation, in part-time work. Sudden death while travelling in 1959
57	183/105	1958	Hypertension for 10 years, angina pectoris for 6 months, left cardiac enlargement. Free from symptoms after Beck operation, in full-time work. Sudden death while travelling in 1959.

4 Is the exercise tolerance improved postoperati ely

5 Is the improvement such as to increase the patient fitness for work?

6. Is life expectancy increased by the operation

Unlike Beck, I have no control series of patients who declined surgical treatment since the operation was accepted by all for whom t was advised. Nevertheless I believ that satisfactory answers to the bove questions can be deduced from the results in my series. All the patients were followed up for at least six months after the operation. In 30 cases the follow-up period was two t four years. Six patients d ed during the observation time five of fresh manifestations of coronary disease and one of ntercurrent bleeding from a peptic ulcer (table II)

The patients own appraisal of their postoperative state is shown in table III (A patient who died of a fresh infarction four days after the operation is not ncluded in this table.) Thirty two patients (75 %) reported freedom or great relief from symptoms. This is less than the figure stated by Brofman (12) (90 %) but should be regarded in the light of the figures for preoperative disablement (80 % in my series and 45 % in Brofman s) The nitrite requirements were ppreciably reduced after operation (table IV) Sixteen patients no longer use nitrites and the mean consumption for the series as a whole fell to one-third of the preoperative figure. The exercise tolerance on bicycle ergometer improved in 25 patients. In general the increase was modest but definite.

Table I Preoperative maximum exercise tolerance in 45 patients who underwent Beck I operation

Kpm/min. for 6 min.	No. of pat.
< 200	11
> 200 — < 300	17
> 300 — < 400	6
> 400 — < 500	5
> 500 — < 600	5
No test	1
Total	45

cent of the dogs which were not protected by a Beck operation but in only 26 per cent of dogs which had undergone this operation.

2 The surface area of the resultant infarcts was 60 to 70 per cent less after the Beck operation than in the controls.

3 The backflow from the distal stump of a severed circumflex artery was twice as great in the Beck treated dogs as in the controls.

In 1956 a follow up study of the first 185 patients who were operated on by Beck for coronary sclerosis was published (12) In 137 consecutive cases in which the observation period ranged from 6 months to 5 years the long term mortality was 13 per cent, as compared with 30 per cent in a control series of patients who had declined the operation. Of the surviving patients, 90 per cent reported complete or appreciable relief from preoperative angina pectoris At the time of follow up 90 per cent were fit for work in contrast to 45 per cent prior to the Beck operation.

Material

In order to obtain personal experience of the clinical value of the Beck operation, collaboration was established in 1957 between the medical department of this hospital and Professor V O Björk, head of the Thoracic Surgery Clinic in Uppsala. At the time of the follow up study (October to November 1961) about 70 patients had undergone the Beck I operation in Uppsala (performed by V O. Björk or A. Hallén) Fifty of them had been referred from Västerås Hospital In 45 cases the postoperative observation period was more than six months. These cases constitute the clinical material of the present report.

In some cases partial ligation of the coronary sinus was omitted for technical reasons. Thrombo-endarterectomy preceded the Beck procedure in one case

The series consisted of 43 male and 2 female patients, 12 were over sixty and 13 under fifty years of age. All had a long history of severe angina pectoris. In the majority of cases it had lasted for more than 3 years. Thirty-six of the patients were unfit for work at the time of the operation. Nineteen cases had been disabled for more than one year One or more episodes of cardiac infarction had occurred in 29 cases. The preoperative exercise tolerance on bicycle ergometer was low (table I) On the whole, therefore, the patients presumably were in poorer condition than those reported by Brofman (12) from Beck's clinic. Thus, 80 % of the present series were totally incapacitated at the time of operation the corresponding figure in Brofman's series was only 45 % Both before and after the operation the patients from this hospital were ordered a dietary regimen in which animal fats were restricted. Anticoagulant treatment according to the principles suggested by Borchgrevink (11) and Waaler (20) was used in suitable cases.

Results

In assessing the value of the Beck operation I have primarily considered the following questions.

1 What is the overall surgical mortality? (The primary surgical mortality was nil)

2 How does the patient judge the result?

3 Does the need for nitrites diminish after the operation?

Table II Deaths during the postoperative observation period

Age (yrs)	B.P. (mm Hg)	Beck op.	Remarks
62	230/120	1958	Malignant arteriosclerosis, albuminuria, left cardiac enlargement. Advised against operation, which was performed at his urgent request. Died 4 days later of fresh infarct.
43	240/130	1958	Malignant arteriosclerosis. Cerebral haemorrhage 1954. Peet operation 1957. Cardiac infarction 1958. Left cardiac enlargement. Greatly improved by Beck operation. Died of gastric haemorrhage in 1960
58	230/140	1958	Malignant arteriosclerosis, lef cardiac enlargement. No relief from Beck operation. Died of cardiac infarction in 1959
54	Normal	1959	Progressive angina pectoris for 8 years. Alcoholic. Subjective improvement after Beck operation. Died of cardiac infarction in 1960.
63	180/110	1958	Severe angina pectoris for 16 years, blood cholesterol 620 mg/100 ml. Ligation of internal mammary artery 1956, little improvement. Greatly improved by Beck operation, in part-time work. Sudden death whil travelling in 1959
57	165/105	1958	Hypertension for 10 years, angina pectoris for 6 months, left cardiac enlargement. Free from symptoms after Beck operation, in full-time work. Sudden death while travelling in 1959.

4 Is the exercise tolerance improved postoperatively

5 Is the improvement such as to increase the patient s fitness for work?

6 Is life expectancy increased by the operation?

Unlike Beck, I have no control series of patients who declined surgical treatment, since the operation was accepted by all for whom t was advised. Nevertheless I believ that satisfactory answers to the abov questions can be deduced from the results in my series. All the patients were followed up for t least six months after the operation. In 30 cases the follow-up period was two to four years. Six patients died during the observation time, five of fresh manifestations of coronary disease and one of intercurrent bleeding from a peptic lcer (table II)

The patients own appraisal of their postoperative state is shown in table III (A patient who died of a fresh infarction four days after the operation is not ncluded in this table.) Thirty-two patients (75 %) reported freedom or great relief from symptoms. This is less than the figure stated by Brofman (12) (90 %) but should be regarded in the light of the figures for preoperative disablement (80 % in my series and 45 % in Brofman s) The nitrite requirements were appreciably reduced after operation (table IV) Sixteen patients no longer use nitrites and the mean consumption for the series as a whole fell to one-third of the preoperative figure The exercise tolerance on bicycle ergometer improved in 25 patients. In general the increase was modest but definite.

Table III Patient s own evaluation of results from 44 Beck I operations

Observation period (yrs)	Report at follow-up			
	No symptoms	Great improvement	Some improvement	No improvement
/ —1	3	7	3	1
1—2	1	5	1	3
2—4	7	7	0	1
Dead	1	1	1	2
Total	12	20	5	7

Table IV Consumption of nitrites by 45 patients who underwent Beck I operation

	No of tablets/day				
No. of patients	0	1—5	6—10	11—20	> 20
Before op.	0	5	27	10	3
At follow-up	16	21	3	3	0

The important question of what the operation implied for the patient s ability to be economically productive is answered in table V At the time of operation only nine patients were fit for full-time or part time work. At follow up examination 23 were employed full time and 3 part time.

The influence of the Beck operation on life expectancy was assessed by deter mining the "force of recurrence in the 24 patients who had had a single episode of infarction (primary infarction) preoper atively The value then obtained was compared with that stated by Blomqvist et al. (10) (table VI) The "force of re currence in these 24 patients was only a third of the figure in Blomqvist s series

Table V Fitness for work after Beck I operation

Observation period (yrs)	Fitness for work			Dead
	Full-time	Part time	Unfit	
/ —1	8	2	4	3
1—2	4	0	6	2
2—4	11	1	3	1

Table VI Incidence of recurrent infarction after Beck I operation (In cases with one infarction before operation)

	Yrs of exposure to risk	No. of recurrences	"Force of recurrence
Biörck et al. (320 ♂ < 60 yrs)	1,269	61	4.8
Biörck et al. (300 ♂ > 60 yrs)	832	37	4.4
24 Beck-treated patients (18 ♂ < 60 yrs, 5 ♂ and 1 ♀ > 60 yrs)	129	2	1.6

May 1st 1963 163, 3 1.8.

Conclusions

The Beck I operation can give gratifying results In many cases of angina pectoris it is followed by considerable amelioration of symptoms restoration of earning capacity and possibly increased life expectancy The ideal operation in coronary sclerosis is, of course, thrombo-endarterectomy but this is almost always precluded by the extent and severity of the arteriosclerotic changes. Only one of the cases in my series was suitable for thrombo-endarterectomy and this was supplemented by a Beck operation In my opinion a Beck procedure should always accompany thrombo-endarterectomy Even if coronary angiography and surgical exploration reveal only a localized atheromatous process,

the basic atherosclerosis is essentially progressive. Coronary angiography was performed in 19 of the cases in the present series. Its value lies mainly in the preliminary information it can give to the surgeon when operation is contemplated.

Contraindictions to the Beck operation are advanced arteriosclerosis, malignant hypertension and dilatation of the left ventricle due to myocardial damage. In such cases the operation should not be advised other than as a last resort on humanitarian grounds, since the inevitable progression of the disease makes the prognosis poor

Experience at this hospital of the medical results of the Beck operation tallies with Beck's own observations and is sufficiently encouraging to warrant increased use of the method and follow-up of the results.

Summary

Forty five patients who had undergone a Beck-I operation for severe angina pectoris were followed up for periods ranging from 6 months to 4 years.

In 29 cases there was a preoperative history of infarction and at the time of operation 80 per cent of the series were unfit for work. Most of these patients had been incapacitated for long periods. The follow-up findings are presented.

1 The primary surgical mortality was nil.

2. The subjective result was reported as very satisfactory by 32 patients (75 per cent) 12 of whom were asymptomatic.

3 The average consumption of nitrites at the time of follow-up was one-third of the preoperative figure.

4 The exercise tolerance in bicycle ergometer tests showed slight but definite increase in 25 cases.

7 [illegible]

5 At the time of operation only 9 patients were fit for work. At follow up 23 patients were in full-time and 3 in part time employment.

6 The "force of recurrence (number of second infarctions per *100* patient years of exposure to risk) in the cases with primary infarction prior to the Beck operation was one third of the figure found by Biörck et al in a series without surgical treatment.

7 Thrombo-endarterectomy was possible in only one case and was supplemented by a Beck I operation. Because of the progressive nature of the underlying atherosclerosis, this operation should routinely accompany thrombo-endarterectomy

8. Coronary angiography was performed in 19 cases. It is of limited value in selecting patients for Beck operation, but can considerably assist the planning of surgery in ischaemic disease of the heart.

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From the Second Department of Internal Medicine (Head K. Lundbæk, M. D.)
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Kommunehospitalet, Aarhus University School of Medicine, Aarhus, Denmark

Disappearance of Waxy Exudates in Diabetic Retinopathy During Administration of p-Amino-salicylate (PAS)

By

V E[illegible], H J Jensen and K. Lundbæk

At least two types of exudates can be distinguished in diabetic retinopathy the small, white or yellow "waxy" exudates usually regarded as characteristic of this condition, and another kind of larger cloudy white or greyish exudates, which we have found to make up about one third of all exudates in diabetes. The waxy exudates are positively correlated to the level of serum cholesterol (9)

van Eck reported that waxy exudates may disappear in patients kept on a low fat diet (8)

Riska has shown a decrease of serum cholesterol during treatment with para-amino-salicylate (PAS) of patients with tuberculosis of the lungs (13) and Tygstrup et al. have shown that the same occurs in hypercholesterolemic patients (15)

The following is a short report of an experiment in which 4 diabetic patients with waxy exudates were observed during the administration of PAS.

Submitted for publication January 17 1963.

Methods

Four patients were selected who had diabetic retinopathy with small areas of waxy exudates which were distinctly observable by ophthalmoscopy and easy to photograph. Visual acuity was normal in all patients.

The patients were on a diet with reduced carbohydrate content, but without restrictions as to the amount or kind of fat. All the patients were on insulin NPH 50.

Ophthalmoscopy was performed and retinal photographs obtained once a month.

For three consecutive days before starting the experiment and once every 1—2 month during administration of PAS the following determinations were performed serum cholesterol, phospholipids and total lipids (14) PBI (2), and basal metabolic rate. The neck was examined regularly to see if any enlargement of the thyroid gland had occurred.

Case reports

Case 1 A 64-year-old woman with moderate diabetes mellitus of 12 years duration. She was well controlled on 16 units of NPH 50-insulin per day Ophthalmoscopy revealed

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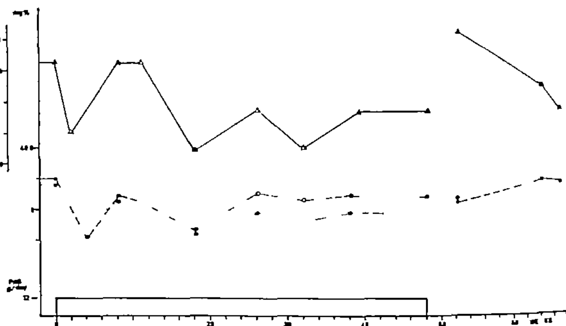


Fig 1 a

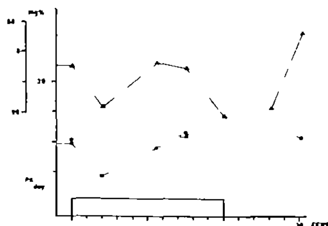


Fig. 1 b

several "microaneurysms moderate phlebopathy hemorrhages and several waxy exudates in both eyes.

The patient was given "Ca Pasido granulate 4 g three times daily (10 g of para amino-salicylate-Ca) for eleven months.

Fig 1 a shows the effect of the treatment on serum cholesterol, phospholipids and total lipids.

The cholesterol level was reduced by 20—30 % for 20 weeks. Afterwards it rose somewhat, but did not return to pre treatment level. The phospholipid curve follows the cholesterol curve. Total lipids were reduced by about 20 % PBI rose from between 5 and 6 to a level of 7 to 10 γ %.

After cessation of the PAS-treatment all the values are seen to return towards the pre-treatment values.

The waxy exudates began to shrink after 1 month of treatment. During the next 3—4 months they seemed to dissolve slowly and had disappeared after 11 months of treatment.

Four months after cessation of PAS-treatment new waxy exudates had appeared in the eye-grounds.

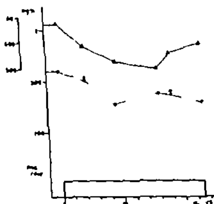


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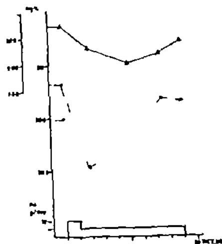


Fig. 1 d

Fig. 1 a—d. Serum cholesterol (O——O), phospholipids (● ●) and total lipids (Δ——Δ) during administration of PAS in 4 long-term diabetic patients.

Case 2. A 58-year-old man with moderately severe diabetes of 14 years duration. At the time of investigation he was reasonably well controlled by two daily injections of 36 and 12 units of NPH 50-Insulin. Ophthalmoscopy showed moderate diabetic retinopathy with sharply defined exudates.

The patient was given PAS 4 g 3 times daily for 5 months.

Fig. 1 b shows a fall in the serum cholesterol values. Phospholipids do not change but the total lipids fluctuate.

After cessation of treatment there is a rise in phospholipids and in total lipids, but not in cholesterol.

During the first 3 months no definite change of the exudates were noted, but after 4 months the typical yellow glossy exudates had changed into grey dull colour and 2 months later the exudates had disappeared. During 4 months of observation after discontinuation of the PAS-treatment no relapse had occurred at the site of the former exudates. I other areas, however new waxy exudates had appeared.

Case 3. A 41-year-old man with moderately severe diabetes mellitus of 12 years duration. He was fairly well controlled on NPH 50-Insulin, 36 plus 16 units a day. Ophthalmoscopy revealed mild diabetic retinopathy with a small group of waxy exudates in the right retina.

The patient was put on treatment with PAS, 4 g 3 times daily for 6 months.

Fig. 1 c shows a definite and sustained fall in the three lipid fractions. PBI rose from about 4 to about 6 γ %.

After 1 month of treatment an unmistakable decrease of the exudates was evident, one month later only 3 small exudates remained, and after a further 3 months of treatment the last exudates had disappeared (fig. 2)

Case 4. A 35-year-old man with severe unstable diabetes of 24 years duration. Insulin 44 plus 16 units NPH 50. Ophthalmoscopy moderate diabetic retinopathy with several small areas of confluent waxy exudates.

The patient was put on PAS-treatment, but the dose had to be reduced to 6 g per day on account of nausea and diarrhoea. This dose was well tolerated for seven months.

Fig. 1 e shows the results of the chemical determinations. After the short period when the patients took 12 g of PAS per day the serum cholesterol and total lipid had fallen. During treatment with 6 g per day the total lipid stayed at a low level. Serum cholesterol rose again, but did not return to the values observed before treatment. The phos-

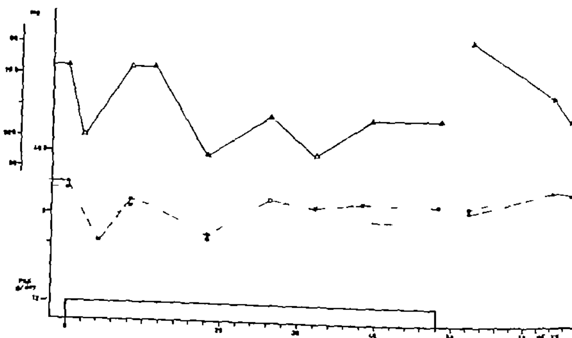


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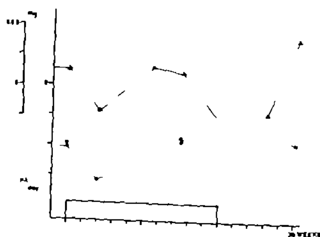


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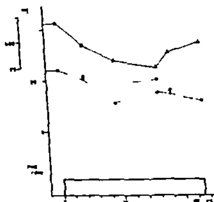


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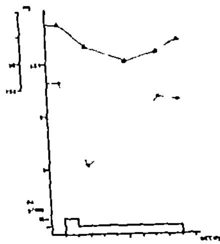


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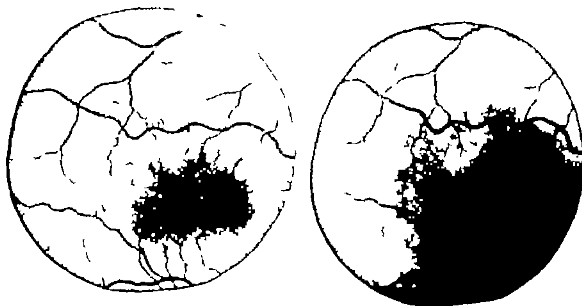


Fig 2. Disappearance of waxy exudates after 6 months of PAS (case no. 5) (Left before; right after)

pholipid curve follows the serum cholesterol curve.

PBI rose from a level of about 6 to values between 7 and 9 γ %.

During the first months of treatment the exudates showed only small changes. After 5 months, however a definite decrease in number and size of exudates was evident.

Discussion

Pasido-calcium-granulate in a dose of 12 g a day (10 g of calcium para aminosalicylate) was well tolerated by three of the patients, but a fourth could take only 6 g

The control of the diabetes was not influenced.

During the PAS-treatment the cholesterol content of the blood was reduced to a lower level. This finding confirms the results of Riska (13) and Tygstrup et al. (15) A marked reduction of total lipids of the blood was seen in three of the patients. Three of the four patients showed a fall in phospholipid

PAS has been shown to possess a weak antithyroid effect (7 12) but studies of the thyroid function during PAS-treatment have shown no depression of the 24- and 48-hour uptake of iodine (1) Cases of goitre have been reported to occur during the treatment with 5—15 g a day (5) but the incidence is very low (4) However as a possible change in thyroid function would influence the lipid levels of the blood we decided to include, in this study regular determinations of PBI and basal metabolic rate as well as measurement of the circumference of the neck.

The basal metabolic rate remained unchanged in two of the patients. In one patient (case no. 1) there was perhaps a slight rise. In the fourth patient it was not possible to obtain reliable curves. There were no clinical signs of a change in thyroid function and none of the patients developed struma.

A considerable and sustained rise in the PBI was seen in the three cases examined. This was a surprise, but eventually it turned out that the coloured coating of the PAS preparation used in

this study contained an organic iodine compound (10). This problem will be dealt with in another publication.

The waxy exudates decreased in all the patients in the course of one to four months. In three patients they disappeared entirely while only partial dissolution occurred in the fourth patient who was on a lower dose of PAS.

According to general clinical experience waxy exudates are among the most persistent features of diabetic retinopathy. When present they are seen on each examination, month by month and year by year unchanged or increasing in number. It is not possible to state that waxy exudates never disappear spontaneously but it seems very unlikely that this should have been the case in all these four patients.

The retinal changes observed in this study during PAS-treatment are the same as those observed by van Eck during treatment with a low fat diet (8). Recently King and Dobree (9) have seen disappearance of waxy exudates during treatment with a diet rich in unsaturated fatty acids.

These three procedures result in a fall of serum cholesterol. The mechanisms of action are not known, but it is not likely that low fat intake, high intake of unsaturated fat and ingestion of PAS should act in the same way. It is known that waxy exudates are deposits of lipoprotein in deep layers of the retina (5, 6, 16). It seems reasonable to assume that the appearance of waxy exudates is directly related to the content or the physical state of the cholesterol of the blood in diabetes mellitus, and possibly to other lipid fractions as well. This implies that if another procedure were tried, resulting in a fall of serum cholesterol, one would expect to see a diminution of waxy exudates in the retina. The fact that there is a statistical correlation between the serum cholesterol concentration of the blood and the incidence of waxy exudates in diabetic retinopathy also supports this interpretation (9).

The practical importance of treatments which influence waxy exudates is not great. Decrease of vision and blindness in diabetic patients nearly always result from quite other causes, namely vascular proliferations and haemorrhages into the vitreous body.

In rare cases, however, blindness does occur due to exudates spreading out over larger and larger areas of the retina. In such patients it seems justified to try the effect of a diet or a drug that lowers serum cholesterol. It is not certain, of course, that the disappearance of large masses of exudates in blind or nearly blind patients will result in an amelioration of vision.

Summary

The waxy exudates in 4 cases of diabetic retinopathy disappeared during administration of 5—10 g of para-amino-salicylate per day.

This drug causes a fall in the serum level of cholesterol and usually also of phospholipids and total lipids.

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Fig 2. Disappearance of waxy exudates after 6 months of PAS (case no. 3) (Left before right after.)

pholipid curve follows the serum cholesterol curve.

PBI rose from a level of about 6 to values between 7 and 9 γ %

During the first months of treatment the exudates showed only small changes. After 5 months, however a definite decrease in number and size of exudates was evident.

Discussion

Pasdo-calcium-granulate in a dose of 12 g a day (10 g of calcium para aminosalicylate) was well tolerated by three of the patients but a fourth could take only 6 g

The control of the diabetes was not influenced

During the PAS-treatment the cholesterol content of the blood was reduced to a lower level. This finding confirms the results of Ruska (13) and Tygstrup et al. (15) A marked reduction of total lipids of the blood was seen in three of the patients. Three of the four patients showed a fall in phospholipid

PAS has been shown to possess a weak antithyroid effect (7 12) but studies of the thyroid function during PAS-treatment have shown no depression of the 24- and 48-hour uptake of iodine (1) Cases of goitre have been reported to occur during the treatment with 5—15 g a day (5) but the incidence is very low (4) However as a possible change in thyroid function would influence the lipid levels of the blood we decided to include, in this study regular determinations of PBI and basal metabolic rate as well as measurement of the circumference of the neck.

The basal metabolic rate remained unchanged in two of the patients. In one patient (case no. 1) there was perhaps a slight rise. In the fourth patient it was not possible to obtain reliable curves. There were no clinical signs of a change in thyroid function and none of the patients developed struma.

A considerable and sustained rise in the PBI was seen in the three cases examined. This was a surprise, but eventually it turned out that the coloured coating of the PAS preparation used in

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Serum Vitamin B_{12} Determinations and Cytochemical Reactions in the Differential Diagnosis of Acute Leukaemia[1]

By

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A histological differential diagnosis of acute leukaemia is often difficult and sometimes impossible. Since a correct differential diagnosis may be of therapeutic importance (5) supplementary diagnostic tools are desirable. For this reason determinations of the serum vitamin B_{12} and cytochemical examination of the cells in the blood and bone marrow have been studied for their value in the differentiation of the various types of acute leukaemia.

The serum vitamin B_{12} in acute leukaemia has been determined by Beard et al. (3) and Mollin and Ross (14). While the former group reported a frank increase of the serum vitamin B_{12} in "acute myelocytic leukaemia" the latter found such an increase in only a few cases of a series of "subacute or acute granulocytic leukaemia".

Cytochemical examinations have been reported by Hayhoe and Quaglino (7) and Hayhoe (6). They found the alkaline phosphatase score in the neutrophilic granulocytes of peripheral blood to be increased in lymphoblastic leukaemia, to be decreased in myeloblastic leukaemia, and to be intermediate in "acute monocytic or myelomonocytic leukaemia". With the PAS-reaction Quaglino and Hayhoe (15) found no staining in leukaemic myeloblasts, while the cytoplasm in leukaemic lymphoblasts often contained relatively large, distinct PAS-positive granules. Mitus et al. (13) have found a low score of PAS-positive substance in lymphocytic cells in acute lymphocytic leukaemia.

The main purpose of the present investigation was to assess the value of determination of the serum vitamin B_{12}-concentration, the alkaline phosphatase score in neutrophilic granulocytes, and the PAS-reaction in leukaemic blast cells as supplementary methods in the differential diagnosis of untreated acute leukaemia.

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Table I

Case	Diagnosis	Hb (g %)	RBC (/mm³ × 10⁶)	WBC (/mm³ × 10³)	Mononuclear cells in blood smears in % of WBC	Blastcells (myelo-lympho-non-classifiable) in bone marrow smears in % of WBC	Erythroid : myeloid ratio	Platelets (/mm³ × 10³)	Serum B_{12} (pg/ml)	Alkaline phosphatase score	PAS-staining: Diffuse (%)	PAS-staining: Granular score
1	AML	4.0	1.4	149	93	87	0.3/100	21	3 000	0	67	33
2	AML	8.9	3.0	0.8	82	91	32/100	91	330	0	94	128
3	AML	8.1	2.3	4.9	57	60	11/100	60	2,180	3	71	20
4	ALL	3.2	1.3	0.9	96	100	3.3/100	6	670	200	0	51
5	ALL	10.2	3.4	563	98	97	0.3/100	31	700	219	0	46
6	ALL	9.4	3.2	8.1	96	100	0.8/100	52	590	256	0	280
7	UAL	5.5	1.8	1.1	85	85	57/100	55	150	153	23	109
8	UAL	7.9	2.7	7.8	98	98	2/100	27	510	108	0	97
9	UAL	8.5	2.5	103	98	99	1/100	108	9,600	178	0	41
10	UAL	10.7	3.7	4.2	48	80	8/100	106	290	116	0	5
11	UAL	10.0	3.7	2.0	78	38	18/100	130	2,120	159	71	125

Case 2. Male, aged 45 years. The patient had felt tired for 1 month, he had had an infected anal ulceration and fever for 14 days, and during the last week before admission he had had signs of cholangiolitis possibly due to chlorpromazine medication. On admission these symptoms were regressing. At examination he had low-grade fever anaemia, leukopenia with dominance of myeloblasts, and moderate thrombocytopenia. The oral cavity tonsils and pharynx were of normal appearance. Close to the anus he had a small superficial smeary ulcer. Urine analysis revealed nothing remarkable.

Case 3. Male, aged 27 years. For 2 months the patient had had gastric pain, anorexia, and he had lost weight, and for one week before examination he had also had fever. At examination body temperature was normal. The patient was found to be anaemic and thrombocytopenic. The number of white blood cells was normal with a dominance of immature cells of myeloid type. The oral cavity tonsils and pharynx were of normal appearance. The superficial lymph nodes, liver and spleen were not palpable. Urine analysis revealed nothing remarkable.

ACUTE LYMPHATIC LEUKAEMIA

Case 4. Female, aged 32 years. For 1 month this woman had had swelling of the neck, haemorrhagic diathesis, fever and anorexia. On examination she had fever signs of haemorrhagic diathesis, pronounced anaemia and leukopenia with dominance of lymphoblasts and thrombocytopenia. The tonsils were swollen with ulceration on the left side. The oral cavity and pharynx were of normal appearance. The superficial lymph nodes of the neck were enlarged but not tender. The liver and spleen were not palpable. Blood

Material and methods

The clinical series consisted of 11 patients (8 males and 3 females) aged 23 to 57 years, with untreated acute leukaemia.

Histological examination

Smears of peripheral blood and bone marrow were stained by the May-Grünewald Giemsa method. Each smear was first typed provisionally by three of us separately and then definitively by all three in consultation. Classified according to the morphological criteria given in a later section the cases fell into three groups

1) Acute myeloid leukaemia (AML)
2) Acute lymphatic leukaemia (ALL)
3) Unclassifiable acute leukaemia (UAL)

Only cases diagnosed as AML or ALL by all three examiners were classified as such. Cases on which complete agreement was not achieved were assigned to the UAL group. The examiners were unaware of the patients from whom the preparations had derived, of the results of the serum B_{12} determinations, and the results of the cytochemical examinations.

Cytological criteria (mainly according to Lee et al. (11))

Acute myeloid leukaemia (figs. 1 and 2)

Wide variation of cell size, wide variation in the ratio between cell size and amount of cytoplasm, basophilic cytoplasm. Auer bodies and granules in the cytoplasm, wide variation of shape of nucleus — round or indented. Chromatin arranged in a fine, even network. One or more nucleoli. A few isolated promyelocytes and myelocytes.

Acute lymphatic leukaemia (fig. 3)

Cells of about the same size; nucleus large relative to amount of cytoplasm, basophilic cytoplasm, no Auer bodies or granules in cytoplasm, round nucleus sometimes with irregularities in chromatin structure, one or more nucleoli, no myeloid cells in intermediate phase of maturation.

Unclassifiable acute leukaemia (figs. 4 a and b)

All cases in which the leukaemic blast cells were neither of AML or ALL type were assigned to this group. The cytology in this group varied widely. Further details are given in the case reports.

Special studies

The serum vitamin B_{12} was determined by the method of Killander (10) with *Euglena gracilis strain Z* as a test organism. Normal values, 200—900 picograms (pg)/ml serum. Subnormal values 100—200 pg/ml serum. Vitamin B_{12} deficiency < 100 pg/ml serum.

Alkaline phosphatase staining. Peripheral blood smears were stained for alkaline phosphatase by the method of Kaplow (9) as modified by Merker and Heilmeyer (12). Alpha naphthyl-phosphate was used as substrate and "Variaminblausalz" (Hoechst) as diazosalt. Neutrophilic granulocytes were graded according to the score method of Merker and Heilmeyer (12). 50—100 cells were counted, depending on the number of cells in the smear. Normal score 20—100.

PAS-staining was done according to the method of Hotchkiss (8). The PAS-positive substance in the blast cells in the bone marrow was semiquantitatively estimated from the number of diffusely stained cells per 100 cells, and secondly from the granule score according to Mitus et al. (13). 100—300 cells were examined separately by two examiners.

Case reports

Haematological data are summarized in table I.

ACUTE MYELOID LEUKAEMIA

Case 1. Male, aged 46 years. The patient had felt tired for about 6 months. For the previous 14 days he had had inflammation of the middle and external right ear. At examination he had high grade fever, right sided otitis media and externa, signs of haemorrhagic diathesis, anaemia, leukocytosis with dominance of myeloblasts. The number of thrombocytes was normal. A few pustules were seen in the gum. The oral cavity, tonsils and pharynx were otherwise of normal appearance. Superficial lymph nodes in the right mandibular angle and in the groins were *slightly enlarged, but not tender*. The liver and spleen were not palpable. Culture of material from the pustules in the gum gave abundant growth of α-streptococci. Urine analysis revealed nothing remarkable.

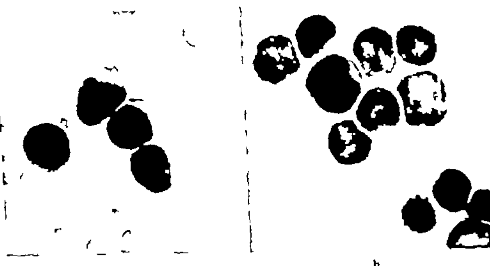


Fig. 4 and b. Unclassifiable acute leukaemia (UAL). T different iews of the same bone marrow smear (patient 8) May-Grünewald-Giemsa. × 1,000.

remarkable. Blood culture gave growth of diphtheroid rods, probably secondary infection. Urine analysis revealed nothing remarkable.

Sternal puncture. Dominance of large reticulum-cell-like blast cells. Abundant plasma cells. Somewhat hyperplastic erythropoiesis. *The cytoplasm* of the blast cells was generally abundant, basophilic, often with clearing around an eccentric nucleus. Some cells contained abundant granules in the perinuclear zone, sometimes vacuoles. No Auer bodies. *The nuclei* were relatively large, round, oval or kidneyshaped. The relationship between the size of the nucleus and the amount of plasma was increased in a few of the smaller cells, but not in the dominating larger cells. The chromatin was stippled or contained formations resembling tufts of hair.

Sometimes condensates were seen, particularly around nucleoli. There were 1 to 6 large, basophilic nucleoli.

Case 8. Female, aged 41 years. For one month the patient had had cold and severe soreness of the throat. During the last 3 weeks the cervical lymph nodes had been swollen, and during the last week she had had pain in the region of the liver. At examination she was febrile, she had anaemia, thrombocytopenia, and swelling of the cervical lymph nodes. The mucosa of the throat was reddened. The oral cavity and the tonsils were of normal appearance. Apart from a few hazelnut-sized, tender lymph nodes in the right and left mandibular angle the superficial lymph nodes appeared normal. The liver and spleen were not palpable. Urine analysis revealed nothing remarkable.

Sternal puncture. Dominance of small to very large blast cells. *The cytoplasm* was generally scanty and basophilic, sometimes with perinuclear clearing. A few cells showed sparse granules and vacuoles. No Auer bodies were detected. *The nuclei* were round, oval or indented. The nucleus plasma ratio was increased. The chromatin was coarse and even but in few cells it appeared condensed. The cells contained 1 to 3 medium-sized nucleoli and bundant mitoses.

Case 9. Male, aged 23 years. For 1 month he had had anorexia, he had lost weight and had had spells of fatigue, gastric pain and fever. At examination he was febrile, anaemic with leukocytosis and moderate thrombocytopenia. No signs of haemorrhagic diathesis were noted. The tonsils were slightly enlarged, but otherwise normal. The oral cavity and the

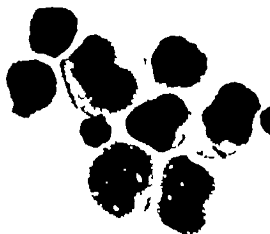


Fig. 1. Acute myeloid leukaemia (AML). Bone marrow smear from patient 2. May-Grünewald-Giemsa. × 1 000.



Fig. 2. Acute myeloid leukaemia (AML). Bone marrow smear from patient 3. May-Grünewald-Giemsa. × 1,000.

culture gave growth of non-plasma-coagulating *Staphylococcus albus*. Urine analysis revealed nothing remarkable.

Case 5. Male, aged 29 years. For 1 month the patient had felt tired and for 3 weeks he had had a haemorrhagic diathesis and general swelling of the lymph nodes. At examination



Fig. 3. Acute lymphatic leukaemia (ALL). Bone marrow smear from patient 5. May-Grünewald-Giemsa. × 1,000.

he had low grade fever; he had petechiae and bruises on the trunk and legs, moderate anaemia, leukocytosis with dominance of lymphoblasts, thrombocytopenia, general swelling of the lymph nodes and splenomegaly. The oral cavity, tonsils and pharynx appeared normal. The liver was not palpable. Urine analysis revealed nothing remarkable.

Case 6. Male, aged 51 years. For 12 days the patient had had a sore throat and tendency to bleeding. At examination he was afebrile and had a markedly swollen right tonsil with subcapsular haemorrhage, and necrosis in the anterior palate. Examination of the blood showed anaemia, thrombocytopenia, normal number of white blood cells with dominance of lymphoblasts. The superficial lymph nodes, liver and spleen appeared normal. Urine analysis revealed nothing remarkable.

UNCLASSIFIABLE ACUTE LEUKAEMIA

Case 7. Female, aged 57 years. For 14 days the patient had had haemorrhagic diathesis, fatigue, anorexia, and probably fever. At examination she was febrile with anaemia, leukopenia with a dominance of blasts, and thrombocytopenia. The oral cavity, tonsils and pharynx appeared normal. The superficial lymph nodes, liver and spleen showed nothing



Fig. 5. PAS-reaction. Acute myeloid leukaemia. Bone marrow smear from patient 2. May-Grünewald-Giemsa. × 1,000.

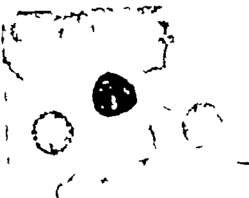


Fig. 6. PAS-reaction. Acute lymphatic leukaemia. Bone marrow smear from patient 5. May-Grünewald-Giemsa. × 1,000.

the widely varying morphological picture in this group.

In none of the three groups was any correlation demonstrable between the serum vitamin B_{12} concentration and the total number of white cells or number of neutrophilic granulocytes in the peripheral blood.

II STAINING FOR ALKALINE PHOSPHATASE (see table I)

Acute myeloid leukaemia

Alkaline phosphatase activity in the neutrophilic granulocytes was, practically speaking, missing in all of the cases. At least one of the patients (No. 1) had an infection and had a high fever

Acute lymphatic leukaemia

All of the patients had a markedly increased alkaline phosphatase score. One (No. 4) might have had sepsis, while none of the others had infection with certainty

Unclassifiable acute leukaemia

In these cases the score was slightly to rather markedly increased, the increase showing no correlation with the morphology. Four of the patients had fever (about 39 C). One (No. 10) had a low fever. In none of these cases could a source of infection be demonstrated with certainty



Fig. 7. PAS-reaction. Unclassifiable acute leukaemia. Bone marrow smear from patient 11. May-Grünewald-Giemsa. × 1,000.

III PAS-STAINING (see table I)

Acute myeloid leukaemia (fig. 5)

Most of the blast cells were diffusely PAS-positive, i. e. the entire cytoplasm

pharynx were of ordinary appearance. The lymph nodes in the groin were slightly enlarged, but not tender. The liver was markedly enlarged and extended down to the iliac crests. The spleen was moderately enlarged. Urine analysis revealed nothing remarkable.

Sternal puncture. Dominance of blast cells of varying size, mostly small. *The cytoplasm* was basophilic, and relatively scanty. No granules or Auer bodies. *The nuclei* were often round, sometimes oval. The nucleus: plasma ratio was generally increased. The chromatin was even and fine. There were 0 to 3 normal-sized nucleoli. Mitoses were abundant.

Case 10. Male, aged 44 years. The patient was first seen after 1 week's history of gastric pain, fatigue, nausea, vomiting and passage of large amounts of urine. On examination he was found to be subfebrile and to have moderate anaemia, leukopenia with only a few blast cells in the peripheral blood, and thrombocytopenia. The oral cavity, tonsils and pharynx were of normal appearance. The superficial lymph nodes, liver and spleen were not enlarged. Urine analysis revealed nothing remarkable.

Sternal puncture. Dominance of blast cells of widely varying size. *The cytoplasm* was basophilic and scanty. Many of the cells showed vacuoles. A few of the cells contained azurophilic granules; no Auer bodies. *The nuclei* were round or oval. The nucleus: plasma ratio was increased. The chromatin net was even, and fine. There were 0 to 3 basophilic nucleoli.

Case 11. Male, aged 50 years. The patient had been under observation for 9 months because of rightsided pleural exudate of unknown origin. During the last few months he had had fever and the X-ray changes on the right side had increased. There was no evidence for tuberculosis. He was admitted to the Department of Chest Surgery with a suspected pulmonary tumour. The lower lobe of the right lung was removed. Histological examination of the operative specimen showed non-specific bronchopneumonia of varying age. In the later course the patient had fever but without demonstrable infection. He was admitted to the Department of Medicine for further study. At the time of the examination the patient was moderately anaemic. The thrombocyte count and the white blood cell count were subnormal. The cervical lymph nodes were enlarged. The liver was also slightly enlarged, but the spleen could not be palpated. Urine analysis revealed nothing remarkable. Blood culture gave no growth. Culture of the sputum gave growth of plasma-coagulating *Staphylococcus aureus* and coliform rods.

Sternal puncture. Dominance of blast cells, usually large and resembling reticulum cells. *The cytoplasm* was often rather abundant. Scattered granules were seen; no Auer bodies. *The nuclei* were round and sometimes kidney-shaped. The nucleus: plasma ratio varied. The chromatin was even and fine. 1 to 4 large, pale nucleoli were seen. Plasma cells were fairly numerous.

Results

I. SERUM VITAMIN B_{12} (see table I)

Acute myeloid leukaemia

In two cases (No. 1 and 3) the serum vitamin B_{12} concentration was increased while in one (No. 2) it was normal. No substantial differences were found between the morphological picture of the bone marrow in these three cases. The patient with the normal serum vitamin B_{12} had, however, a higher erythroid: myeloid ratio than the other two and leukopenia of the peripheral blood.

Acute lymphatic leukaemia

The serum vitamin B_{12} was normal in all three cases.

Unclassifiable acute leukaemia

In one case (No. 7) belonging to this group the serum vitamin B_{12} concentration was subnormal. In two (No. 8 and 10) it was normal and in two (No. 9 and 11) it was frankly increased. No correlation could be detected between the concentration of the serum vitamin B_{12} and

In the present investigation the PAS-reaction varied within each group. Granules were demonstrated in all the cases but the number of cells with granules varied widely as did the number and shape of the granules in the cells. Low and high scores occurred in all three groups and gave no clear-cut diagnostic clues. A relatively large number of the blast cells stained diffusely with PAS in AML but not in ALL. The blast cells stained diffusely in some cases of UAL, but not in others, without any definite correlation with the morphological picture.

Our results differ from those of Quaglino and Hayhoe (15) according to which the myeloblasts in myeloblastic leukaemia are PAS-negative, the monocytes in monocytic leukaemia diffusely PAS-positive, and the lymphoblasts in lymphoblastic leukaemia partly granulated, PAS-positive. Hayhoe (6) believes these differences in the PAS-reaction to be of differential diagnostic value. However Wachstein (18) Gibb and Stowell (4) and Ackermann et al. (1) showed that in acute myeloblastic leukaemia myeloblasts may contain PAS-positive substance, i. e. a finding also made in the present investigation.

In the differentiation between myeloid and lymphatic acute leukaemia staining with PAS appears to be of questionable value. It is possible that the absence of diffuse staining may argue for ALL.

Judging from an analysis of results obtained by previous authors and those described here, neither determination of the concentration of vitamin B_{12} in the serum, nor staining for alkaline phosphatase activity nor staining with PAS is by itself of any value in the differentiation of morphologically obscure cases of acute leukaemia. We therefore tried to find out whether a combination of the three examination methods might increase the possibilities of distinction between the myeloid and lymphatic types of acute leukaemia. Our material is small, and will therefore not permit any generally valid conclusions. It appears, however that the finding of a high serum vitamin B_{12} concentration in association with low alkaline phosphatase score in the neutrophilic granulocytes, and the occurrence of both diffuse and granulated PAS-staining in the leukaemic blasts argue for acute myeloid leukaemia, while a normal concentration of the serum vitamin B_{12} high alkaline phosphatase score and absence of diffuse PAS-reaction of the leukaemic blast cells might argue for acute lymphatic leukaemia. But none of the cases in the unclassifiable acute leukaemia group satisfied either group of criteria. Of the two cases with a high serum vitamin B_{12} concentration in this group (No. 9 and 11) one showed no diffuse reaction with PAS and the alkaline phosphatase score was high in both. The cases could thus not be classified as acute myeloid leukaemia. The cases with a normal serum vitamin B_{12} (No. 8 and 10) showed no diffuse PAS-reaction but only a slightly increased alkaline phosphatase score, i. e. the results were not typical of acute lymphatic leukaemia. The patient with the subnormal vitamin B_{12} concentration (No. 7) had a moderately increased alkaline phosphatase score in the leukaemic blast cells, but the PAS-reaction in these cells showed the diffuse pattern, which argues against acute lymphatic leukaemia.

Thus, determination of the vitamin B_{12} concentration in serum, staining for alkaline phosphatase in neutrophilic granulocytes of peripheral blood and PAS-

stained faintly red In addition a varying number of cells (8—70 per cent) contained distinct granules.

Acute lymphatic leukaemia (fig 6)

No diffuse staining occurred Distinct granules of varying size were seen in different numbers of cells (20—90 %) Some cells contained a larger accumulation of PAS-positive substance

Unclassifiable acute leukaemia (fig 7)

Diffuse staining occurred in two cases (No 7 and 11) All of the cases showed granules of varying type as in AML and ALL (5—75 %) No definite correlation was found between PAS-staining and morphology

Discussion

In a series consisting of 20 cases of acute leukaemia Beard et al. (3) found a frank increase of the serum vitamin B_{12} concentration in *all* the six cases of "acute myelocytic leukaemia Since the values found in the other acute forms of leukaemia were normal the investigators assumed that knowledge of the concentration of the serum vitamin B_{12} might be useful in the differential diagnosis of acute leukaemia. In the present small series it was found that in three cytologically typical cases of AML the serum vitamin B_{12} concentration was increased *in two* In all three cases of ALL the serum vitamin B_{12} concentration was normal In the UAL group it was normal or increased without any certain correlation with the morphological picture. Our results tally best with those found by Mollin and Ross (14) in thirty-six cases of acute leukaemia. In eleven of their cases in which the bone marrow was filled with cells that had not matured beyond the myeloblast or lymphoblast stage the serum vitamin B_{12} concentration was normal. It was also normal in those patients in whom the blast cells "showed evidence of early differentiation towards the granulocytic series" Of the fifteen clearly myeloid cases, only five had an increased concentration of the serum vitamin B_{12}. Raccuglia and Sachs (16) reported similar results.

It would thus appear that an increased serum vitamin B_{12} concentration might argue for myeloid and against lymphatic acute leukaemia while a normal serum vitamin B_{12} concentration is of no differential diagnostic value.

Like previous investigators, e. g., Hayhoe (6) we found the alkaline phosphatase score to be low in AML but high in ALL. In the cases of UAL the values were relatively high. Since infection is accompanied by an increase of the alkaline phosphatase score (7 17) and since infections are common in acute leukaemia one might suspect that the results obtained in the present investigation were influenced by infection. One of the patients in the AML group was frankly infected but the score was nevertheless nil. One patient with ALL might have had sepsis, but the other two had no definite signs of infection, yet the score was definitely increased. This might indicate that the low alkaline phosphatase score in AML is not influenced by infection and that the higher score in ALL is not due to an infectious process. If this assumption be correct, the alkaline phosphatase score might be of value in the differential diagnosis of acute leukaemia. However we found it of no help in our UAL cases, in all of which the score was increased without reaching the score values in the ALL group.

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Coexisting Hyperthyroidism and Hyperparathyroidism

By

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Up to 1957 only three cases of hyperthyroidism associated with hyperparathyroidism due to histologically confirmed parathyroid adenoma had been reported (28, 33, 35). Since then, 7 more cases have been observed (7, 15, 17, 19, 24, 25 a). One case has been reported in which hyperparathyroidism appeared 1 1/2 years after thyroidectomy for hyperthyroidism (18). In addition, there have been reports of several similar cases in which, however, no detailed evidence of hyperparathyroidism is given (2, 3, 5, 8, 10, 21, 32).

In two cases with hyperthyroidism and hypercalcaemia seen during the last three years at the First Medical Department of Helsinki University parathyroid adenoma was found at operation. The case records will be presented.

Case reports

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Eleven untreated cases of acute leukaemia in adults were studied by serum vitamin B_{12} determinations, staining of the alkaline phosphatase activity of neutrophilic granulocytes of peripheral blood and PAS-reaction in leukaemic blast cells from the bone marrow. The results were compared with impressions obtained by studies of May Grünewald Giemsa stained smears of the peripheral blood and the bone marrow.

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In a group of morphologically unclassifiable cases of acute leukaemia the investigations remained with a mixed pattern adding no further help in the differential diagnosis.

Acknowledgement

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Coexisting Hyperthyroidism and Hyperparathyroidism

By

P Tornti and B.-A. Lamberg

Up to 1957 only three cases of hyper thyroidism associated with hyperparathyroidism due to histologically confirmed parathyroid adenoma had been reported (28, 33 35) Since then 7 more cases have been observed (7 15 17 19 24 25 a) One case has been reported in which hyperparathyroidism appeared 1 1/2 years after thyroidectomy for hyperthyroidism (18) In addition, there have been reports of several similar cases in which, however no detailed evidence of hyperparathyroidism is given (2, 3, 5 8, 10, 21 32)

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By

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Table I Case 1

Period	Body weight (kg)	Urinary output (ml)	Serum Ca (mg%)	Serum P (mg%)	Urinary calcium excret. (mg/day)	Urinary phosph. excret. (mg/day)	Serum alk. phosph. (KA units)	PBI (μg%)	Serum cholest. (mg%)	^{131}I test
10/7—4/8 1959 Before ^{131}I treatment	54	600–1,350	–	–	–	–	6.6	13.0	146	E_1 7% E_2 1%
3/9–2/10 1959 Preoperatively (Med. Dept.)	56–58	1 100–1,930	10.8–11.8	3.9	584	–	6.5	8.3	187	–
3/10–13/10 1959 Preoperatively (Surg. Dept.)	62	950–1 800	10.6	3.5	432	–	–	–	–	–
Operation 14/10										
14/12–18/12 1959 Postoperatively	73.5	200–800	8.6	–	161	–	–	–	–	–

E_1 = Urinary excretion of radioactive iodine, 1st day
E_2 = Urinary excretion of radioactive iodine, 2nd day

calcium and inorganic phosphate were not determined. The serum protein bound iodine was 13 μg/100 ml and the serum cholesterol 146 mg/100 ml, the BMR + 85. The urinary excretion of radioactive iodine was 7 % on the first day and 1 % on the second.

Because of the evident clinical hyperthyroidism the patient was given 14 mc of radioactive iodine on July 29th, 1959. The gastric ulcer was treated with spasmolytic and neutralising drugs. During the three weeks in hospital the abdominal distress totally disappeared and the hyperthyroidism improved considerably.

The patient was readmitted on Oct. 2nd, 1959. Soon after discharge from hospital th abdominal distress recurred and the sweating and thirst increased. The weight on readmission was 57 1/2 kg and the physical findings were similar to those on the first admission. The ESR was 35—53 mm/hour. Hb 10 g/100 ml, cholesterol 187 mg/100 ml, serum calcium 11.8 mg/100 ml and inorganic phosphorus 3 9 mg/100 ml. The daily urinary calcium excretion was 584 mg. Dail urinary volumes were about 2 litres. In the water deprivation test the specific gravity increased to 1 026. The patient was given a combined treatment with carbimazole (30 mg a day) and potassium iodide (5 % solution 10 drops twice daily) as pre-operative medication for the persisting hyperthyroidism. After two weeks of treatment the patient appeared euthyroid and was transferred to the surgical department for subtotal thyroidectomy because of persistent hyperthyroidism and suspicion of simultaneous hyperparathyroidism.

The operation was performed on Oct. 14th, 1959. Subtotal thyroidectomy was carried out and partly nodular thyroid tissue was removed. On the left side of the neck outside the thyroid gland a nodule was detected and removed.

Histological examination of the nodule outside the thyroid revealed parathyroid tissue with acinary and cystic structures, remarkable loss of fat and with nuclear hypertrophy. The cells were all of the chief cell type (fig. 1).

The changes in the thyroid tissue were consistent with hyperfunction in a nodular goitre

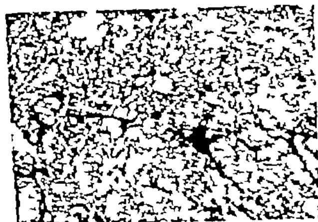


Fig. 1 Microscopical section of the nodule outside the thyroid removed in patient no. 1 revealing an acinary and cystic structure, loss of fat and nuclear hypertrophy

The postoperative course was uneventful. Three days after the operation the serum calcium was 10.2 mg/100 ml and the daily urinary excretion 374 mg. The patient was readmitted to the surgical department on Dec. 14th, 1959 for follow-up study. During the postoperative period his general condition had gradually improved. He was euthyroid, the fatigue, sweating and thirst had disappeared. His body weight was 73.5 kg and the heart rate 70/min. Daily urine volumes were below 800 ml and the calcium excretion was 161 mg. The serum calcium was 8.6 mg/100 ml. The urine sediment had been normal all the time and no proteinuria was detectable; the specific gravity of the urine was 1.026.

Case 2. Wife of an agricultural worker, 61. She was admitted on April 12th, 1961 for upper abdominal pain and vomiting of 2 months duration. Recurrent urinary infections had been observed 7 to 8 years before. The gallbladder had been removed six years previously for gall stones. One year after the operation a duodenal diverticulum was removed and one year later a cyst from the lower pole of the right kidney. The patient was of pycnic stature, height was 162 and weight 70 kg. She suffered no acute distress. Her hands were moist and showed a coarse tremor. The thyroid gland appeared to be of normal size and without nodules. On auscultation there was a grade I holosystolic murmur which was regarded as accidental, the heart rate was 88/min. and the blood pressure 155/100. The pulmonary findings were normal. There was marked tenderness in the right epigastric area but no masses were palpable. In intravenous cholangiography the bile duct appeared to be somewhat dilated but showed no stones, and the upper gastrointestinal tract was normal on X-ray examinations. The ESR was 28 mm/hour, RBC 3.62 mill./mm³, the Hb was 10.8 g/100 ml and the MCH 30. The WBC was 3,500/mm³ and the differential count normal. The alkaline phosphatase was 11.3 King Armstrong U. and the serum and the urinary amylase values within normal limits. The fasting blood glucose was 103 mg/100 ml and the oral glucose tolerance showed a diabetic pattern. There was no glucose or protein in the urine. In the urinary sediment leukocytes were abundant and 5—10 red blood cells were found per high power field. *Escherichia coli* was cultured. During antibiotic treatment (chloramphenicol, streptomycin and streptomycin-penicillin) the epigastric tenderness vanished and the urine sediment became normal. The patient was discharged almost symptom free with the diagnosis of chronic pancreatitis and recurrent pyelonephritis.

She remained symptom free until Sept. 1961 when she began to suffer from increasing fatigue and thirst. There was shortness of breath in addition and she was readmitted to the hospital on Nov. 10th, 1961 because of acute upper abdominal pain and vomiting.

On admission her general condition seemed to be fairly good. The skin was moist and warm, the thyroid gland was normal on palpation, and no nodules were felt. There was swelling in the upper eyelids but no other eye symptom. The body weight was 71 kg. Psychically the patient appeared to be calm

Table II Case 2

Period	Body weight (kg)	Urinary output (ml)	Serum Ca (mg%)	Serum P (mg%)	Urinary calcium excret. (mg/day)	Urinary phosph. excret. (mg/day)	Serum alk. phosph. (KA-units)	PBI (μg%)	Serum cholest. (mg%)	^{131}I-test
14/11–27/11 1961 Before treatment	71.2	800–2,800	12.8–14.0	2.4–2.8	286–368	533–1 062–	13.6	13.81	168	U_{24} 78% E_1 10%
28/11–31/12 1962 Preoperatively	67.9–72.7	1,300–2,300	12.3–13.3	1.7–2.2	222–400–	380–612–	—	—	—	—
1/1–1/2 1962 Preoperatively	77.5	—	10.8–11.1	2.4–3.0	122–214	346–670	10.8	—	—	—
Operation 2/2 1962										
13/2–20/2 1962	78.5	1 450–2,100	8.2–8.9	3.6–3.8	27–32	750–1 024	14.2	—	322	—
21/2–29/3 1962	81.0	700–1,250	8.9–9.6	2.7–3.4	33–50	350–420	15.1	—	358	U_{24} 6% E_1 37% E_2 11%

U_{24} = 24 hour uptake.
E_1 = Urinary excretion of radioactive iodine 1st day
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and balanced, but after two weeks she grew impatient and fearful, and had insomnia and hallucinations. The heart rate was 115/min. the arterial blood pressure 150/90. The ECG was normal. There was some tenderness in the right epigastric area but no masses were felt. On X-ray examination the bone density appeared decreased but there were no osteolytic areas. The findings were similar in the thoracic skeleton, the lumbar spine, the pelvic bones, the long bones and the skull. X rays of the chest revealed nothing abnormal, and no calcifications were detectable in the abdominal area.

The ESR was 30 mm/hour. Hb 12.8 g/100 ml, RBC 4.01 mill./mm³ and WBC 3,300/mm³ with a normal differential count. The alkaline phosphatase 13.6 King-Armstrong U., the total serum proteins 6.2 g/100 ml and the electrophoretic distribution pattern normal. Serum calcium was 13.5 mg/100 ml, inorganic phosphorus 2.5 mg/100 ml. The daily urinary excretion of calcium was continuously above 300 mg. The calcium tolerance test (23) showed a pathological response: there was no increase in the serum inorganic phosphorus, and no decrease in the phosphaturia. The tubular reabsorption of phosphate was 78.9 %, but no standard diet allowing a constant phosphate intake was used. Prednisolone, given for one week (25 mg daily) did not change the serum calcium or phosphorus values or the excretion of calcium or phosphorus in the urine. The serum creatinine was 0.72 mg/100 ml, the creatinine clearance was 80 ml/min., and the phenolsulphophthalein excretion was normal. The specific gravity of the morning urine did not exceed 1.010 and during the water deprivation test it only changed to 1,016. There was no glucosuria or proteinuria, and the urinary sediment was normal on repeated examinations. The daily urinary volume remained below 2 litres. The PBI was 13.8 μg/100 ml and the cholesterol 168 mg/100 ml.



Fig. 2. Microscopical section of the node removed in patient no. 2 revealing adenomatous structure with nuclear hypertrophy and loss of fat and the presence of oxyphil cells in addition to chief cells.

The creatine/creatinine ratio in the plasma was 3.4. The radioactive iodine test showed an uptake of 78 % after 24 hours and the urinary excretion was 10 % on the first day. The PB¹³¹I was 1.9 %/l after 72 hours.

As hyperthyroidism was evident, preoperative treatment with carbimazole (30 mg a day) and potassium iodide (5 % solution 10 drops twice daily) was started. The signs of hyperthyroidism gradually diminished and after three weeks of treatment the patient was largely euthyroid. At that time the serum calcium values decreased at times below 11 mg/100 ml and the tubular reabsorption of phosphate increased to 97.9 %. At that time too the response to the intravenous calcium load was normal. The serum inorganic phosphorus still remained low 2.5 mg/100 ml.

On Jan. 24th, 1962, the patient was transferred to the surgical department for subtotal thyroidectomy and for exploration due to suspicion of hyperparathyroidism. Operation was performed on Feb. 2nd, 1962. The thyroid appeared only slightly enlarged and 35 g were removed. In addition at the bend of the right inferior thyroid artery node with a diameter of mm was detected and removed.

Histological examination of the node revealed nuclear hypertrophy and almost total loss of fat tissue, the changes being indicative of adenomatous structure. The presence of oxyphil cells in addition to chief cells excluded primary hyperplasia of the parathyroid gland (fig. 2).

The changes in the thyroid tissue were consistent with hyperfunction and revealed its structure.

The postoperative course was uneventful and the patient was transferred to the department of medicine on Feb. 14th, 1962 for *further examination. She was in good general* condition and euthyroid, and had no subjective complaints. Hb was 13 g/100 ml, WBC 9 400 mm³ the serum calcium 8.7 mg/100 ml and the inorganic phosphorus 3.6 mg/100 ml. The daily urinary excretion of calcium was 27 mg and that of phosphorus 750 mg.

The patient was readmitted for a follow-up study on March 21st, 1962. The serum calcium was 8 mg/100 ml and phosphorus 3.4 mg/100 ml. The daily excretion of calcium and phosphorus was 50 and 420 mg respectively. The uptake of radioactive iodine was 6 % after 24 hours and the urinary excretion 37 % on the first day and 16 % on the second day. The serum alkaline phosphatase remained elevated being 15.1 King-Armstrong U. According to laboratory data she had evidently hypothyroidism which was later on adequately substituted. The PBI was 3.5 μg/100 ml.

Discussion

The negative balance of calcium metabolism in hyperthyroidism which may result in various types of metabolic bone disease (4 14 30) was originally observed by Aub et al. (1) in 1929 and has more recently been further elucidated (9 16 22, 26, 38, 39) also in a few studies with radioisotopes (12, 29) Hypercalcuria is

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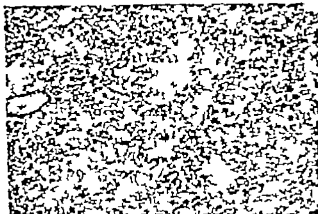


Fig. 2. Microscopical section of the node removed in patient no. 2 revealing adenomatous structure with nuclear hypertrophy and loss of fat and the presence of oxyphil cells in addition to chief cells.

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On Jan. 24th, 1962, the patient was transferred to the surgical department for subtotal thyroidectomy and for exploration due to suspicion of hyperparathyroidism. Operation was performed on Feb. 2nd, 1962. The thyroid appeared only slightly enlarged and 35 g were removed. In addition at the bend of the right inferior thyroid artery node with diameter of 5 mm was detected and removed.

Histological examination of the node revealed nuclear hypertrophy and almost total loss of fat tissue, the changes being indicative of adenomatous structure. The presence of oxyphil cells in addition to chief cells excluded primary hyperplasia of the parathyroid gland (fig. 2)

The changes in the thyroid tissue were consistent with hyperfunction and revealed a microadenomatous structure.

The postoperative course was uneventful and the patient was transferred to the department of medicine on Feb. 14th, 1962 for further examination. She was in good general condition and euthyroid, and had no subjective complaints. Hb was 13 g/100 ml, WBC 9,400 mm³ the serum calcium 8.7 mg/100 ml and the inorganic phosphorus 3.6 mg/100 ml. The daily urinary excretion of calcium was 27 mg and that of phosphorus 750 mg

The patient was readmitted for a follow-up study on March 21st, 1962. The serum calcium was 8 mg/100 ml and phosphorus 3.4 mg/100 ml. The daily excretion of calcium and phosphorus was 50 and 420 mg respectively The uptake of radioactive iodine was 6 % after 24 hours and the urinary excretion 37 % on the first day and 16 % on the second day The serum alkaline phosphatase remained elevated being 15.1 King-Armstrong U According to laboratory data she had evidently hypothyroidism which was later on adequately substituted. The PBI was 3.5 µg/100 ml.

Discussion

The negative balance of calcium metabolism in hyperthyroidism which may result in various types of metabolic bone disease (4 14 30) was originally observed by Aub et al. (1) in 1929 and has more recently been further elucidated (9 16 22, 26 38, 39) also in a few studies with radioisotopes (12, 29) Hypercalciuria is

a fairly common finding in hyperthyroidism but hypercalcaemia seems to be rather infrequent. To our knowledge only 31 cases of hypercalcaemia in hyperthyroidism have been reported (7 11 26 27 28 34 36 38 40 41 42 44) including one case with hypercalcaemic deposits in the kidneys (13) and one with renal tubular acidosis which was thought to have been induced by hyperthyroidism (25) It has, however been stated by Nikkilä and Pitkänen (34) in 1960 from this clinic that slight elevation of the serum calcium may occur in some ten per cent of the cases.

On the other hand the coexistence of hyperthyroidism and histologically confirmed parathyroid adenoma with hyperparathyroidism has been reported in only ten instances. The differential diagnosis of hypercalcaemia in hyperthyroidism may be difficult The clinical symptoms such as nausea vomiting abdominal pain and mental changes are signs of hypercalcaemia but may occasionally occur in masked hyperthyroidism without hypercalcaemia (34) Tubular changes, visible as iso- and hyposthenuria and low specific gravity of the urine, are a consequence of the hypercalcaemia whatever the reason for this may be The alkaline phosphatase may or may not be increased in both conditions. The tubular reabsorption of phosphate (TRP) has been shown usually to be normal in uncomplicated hyperthyroidism (7) with few exceptions even in the presence of hypercalcaemia and on the other hand significantly depressed in hyperparathyroidism whether or not associated with hyperthyroidism. It has recently been shown however that some overlapping occurs between normal subjects and hyperparathyroid patients and that the TRP is greatly influenced by filtered phosphate load and phosphate intake (19 37) Thus, without appropriate control of the phosphate intake it seems difficult to draw conclusions on the basis of a single TRP determination. Then either the absence of hyperparathyroidism in association with hypercalcaemia should ultimately be proved by complete disappearance of the hypercalcaemia or hypercalciuria after adequate treatment of the hyperthyroidism or its presence be demonstrated by histological evidence of a parathyroid adenoma.

The finding of a parathyroid adenoma would seem to prove the case. It has, however been stated that parathyroid adenomata are not always associated with hyperfunction and hyperparathyroidism (6) Hence, hypercalcaemia in a patient with hyperthyroidism in whom a parathyroid adenoma is found at operation does not afford unequivocal proof that the hypercalcaemia was really due to hyperparathyroidism.

In the first patient there was a gradual decrease in the serum calcium and the calciuria during the preoperative treatment and the whole picture could have been considered as due to hyperthyroidism alone At the time of operation when the patient was euthyroid the calciuria was still somewhat elevated and the urinary output higher than after thyroidectomy Three days after operation and removal of the parathyroid adenoma, the serum calcium was still at the preoperative level but the urinary output had diminished The specific gravity of the urine was unexpectedly high, it should have been low irrespective of the reason for the increased calciuria and hypercalcaemia. The fact remains that a parathyroid adenoma was found at operation which was histologically compatible with hyperfunction

In the second patient the previous history of gall bladder disease, abdominal pain and elevated blood alkaline phosphatase suggested pancreatitis on the first admission to hospital, though elevated serum or urinary amylase values could not be detected. On the second admission the findings were similar in addition to evident hyperthyroidism. The recurrence of ill defined abdominal pain made it advisable to examine the possibility of hyperparathyroidism. The laboratory findings and the X-ray findings of the bones were consistent with the presence of bone disease: high serum calcium, low serum phosphorus, increased alkaline phosphatase and demineralisation of the bones. The TRP also was decreased, although its evaluation is not easily made (see above). There was no decrease in serum calcium or calciuria during treatment with prednisolone, which has been taken as indicative of hyperparathyroidism. This seems not to be an absolute criterion however as judged from the very few cases in the literature (19, 20) and one personal observation (not published) in which a decrease has also been observed in that condition. In view of the gradual decrease in hypercalcaemia and calciuria during preoperative treatment in the way that the calcium changes could be interpreted as due solely to the hyperthyroidism. However we think this trend does not necessarily exclude the possibility of parathyroid adenoma, since the elimination of one factor would probably change the pattern in calcium metabolism. The serum phosphorus, however was on the usual hyperparathyroid level and the abrupt change in calcium metabolism after removal of the adenoma at operation would appear to suggest that the adenoma really was hyperfunctioning, since the patient had already been euthyroid for a long time. The alkaline phosphatase remained elevated for a considerable time postoperatively which in other cases has been interpreted as evidence of intense bone formation as a consequence of the previous calcium depletion (28). Curiously in this particular patient the response to the intravenous calcium tolerance test changed to a normal one in the euthyroid state.

Are there then in general any connections between hyperparathyroidism and hyperthyroidism? Opinions seem to be divergent but we agree with Koenig and Gubler (27) that it is difficult to visualise any causal relationship. Furthermore, the frequency of hyperthyroidism in Finland (43) would probably reveal a great number of such cases if such a correlation exists. On the other hand it is interesting to speculate about the simultaneous occurrence of adenomata in the parathyroid and thyroid glands. In the majority of cases of hyperparathyroidism associated with hyperthyroidism, the thyroid has been nodular and there has been either a single nodule or several nodules. Again, it is difficult to trace direct correlations, since over 90 per cent of the goitres in Finland are nodular (31, 43). Yet the possibility remains as the two organs have the same origin, i.e. they are branchial structures.

Summary

Two cases of hyperthyroidism with hypercalcaemia are presented in which parathyroid adenoma was detected on operation. The reasons for regarding the adenomata as hyperfunctioning are discussed. These two cases would be the 11th and 12th of co-existing hyperthyroidism and hyperparathyroidism so far reported.

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Glycogen Content of Leukocytes and Platelets

By

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The peripheral blood is known to contain glycogen chiefly localized in the white blood cells. The question of the distribution of the glycogen between the different types of blood cells has, however, not been settled. Different possibilities therefore for the relative distribution had to be taken into account in a recent study of the relationship between the glycogen content and the periodic acid-Schiff (PAS) reaction of the white blood cells in normal and diabetic subjects (4).

In the present investigation lymphocytes and platelets have been isolated and the glycogen content of these cells has been determined chemically. Values for the granulocytes have been calculated as the remaining part of the total cellular glycogen content in mixed white blood cell suspensions.

Materials and methods

Sampling of the blood

Venous blood samples were obtained from healthy volunteers (medical students, aged 20 to 30 years), mostly before breakfast. A few samples collected postprandially did not differ appreciably from the fasting samples. All cannulas and tubes used for the sampling of blood were siliconized and 250 units of heparin (Vitrum, Copenhagen) was added per millilitre of blood.

Counting and definition of the different types of white blood cells

The white blood cells were counted in triplicate in a Boerker counting chamber: the platelets, in a phase contrast microscope after dilution with a 1 % ammonium oxalate solution (2); the granulocytes and the lymphocytes, separately after dilution with Türk's reagent. The following definitions were used:

White blood cells { platelets; leukocytes { granulocytes; lymphocytes

The monocytes were not counted separately but included in the lymphocyte counts.

Preparation of mixed white blood cell suspension.

Mixed white blood cell suspensions were obtained by sedimentation of the red blood cells in the presence of fibrinogen, as previously described (4). The suspensions were allowed 40—60 min. for sedimentation usually at room temperature (20° C), occasionally at 37° C and 4° C.

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Addendum

During the preparation of this manuscript J Hellström & B. I. Ivemark (Acta chir scand. suppl. 294 1962) have observed three additional cases in their large series of primary hyperparathyroidism.

Results

Glycogen content of the mixed white blood cell suspensions

The mixed blood cell suspensions obtained after sedimentation of the red cells contained granulocytes (3 000—10,000/mm³) lymphocytes (3 000—6,000 /mm³) and platelets (250,000—400,000/ mm³) In most instances the percentage distribution of the different types of cells was approximately the same as that found in blood smears from the same patient. The amount of cellular glycogen in these mixed white blood cell suspensions varied between 50,000 and 100 000 pg¹/mm *The cellular glycogen did not vary with* the sedimentation temperature (4 C, 20° C, and 37° C)

Glycogen content of isolated platelets

The isolated platelet suspensions contained 150,000 to 380,000 platelets/mm³ but no neutrophil leukocytes or lymphocytes. The cell-bound glycogen ranged from 20,000 to 35,000 pg/mm

The mean glycogen content of the platelets, calculated from 12 consecutive experiments, was 0 092 pg per cell. The standard error of the mean was ± 0 005 pg.

Glycogen content of the leukocytes

Lymphocyte suspensions prepared by glass wool filtration contained 1,000—6,500 lymphocytes per mm They also contained platelets (30,000—120 000/ mm³) and usually some granulocytes (0—400/mm³) The elative number of lymphocytes was thus much higher than in the mixed white blood cell suspension, and in the purified suspensions it was as high as 85—99 per cent (of the leukocytes) The cellular glycogen in these preparations was 3,200—15,000 pg/mm

¹ pg (picogram) = 1 × 10⁻¹² gram.

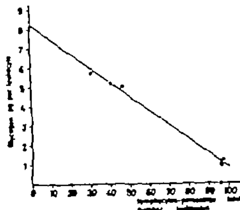


Fig. 1 Glycogen content per leukocyte (after correction for platelet glycogen) as function of the relative percentage of granulocytes and lymphocytes. The values with 30—75 % of lymphocytes have been obtained from mixed white blood cell suspensions, those with 85—99 % *of lymphocytes* from preparations after glass wool filtration. Usually one preparation of each kind was obtained from the same donor

The amount of glycogen per leukocyte was calculated by subtracting the amount present in the platelets from the total amount. In these calculations each platelet was assumed to contain 0 09 pg of glycogen. The amount of glycogen per leukocyte varied widely with the relative number of granulocytes and lymphocytes present (fig 1) Although there was a considerable variation of the values obtained it is clear that the amount of glycogen per leukocyte was substantially higher when the preparation was rich in granulocytes than when it was rich in lymphocytes. The correlation coefficient ( ) between the amount of glycogen per leukocyte and the percentage of lymphocytes in the preparation was — 0.7 Calculation and extrapolation of the regression line to 100 per cent for lymphocytes and granulocytes, respec

Isolation of the platelets

The platelets were isolated by differential centrifugation. 10 ml of heparinized blood was centrifuged at 150 times gravity (Wifug rotor diam. 6 cm, 1 400 r p. m.) for 5 minutes. The supernatant, which contained the platelets, was removed. Resuspension of the buffy coat was carefully avoided. With this method 30—40 % of the original number of platelets was recovered in the supernatant. Centrifugation at lower speed increased the yield of platelets, but also the inclusion of leukocytes, chiefly lymphocytes.

Isolation of the lymphocytes

Two different methods were employed

a) *Isolation of the lymphocytes by glass wool filtration.* This method is based on the tendency of the granulocytes to adhere to glass wool (7 9) and has previously been used in our laboratory for the separation of lymphocytes for *in vitro* cultures (1) Pyrex glass wool fibre No. 3950 (Corning Glass Works, Corning New York, U S. A.) was packed in a glass tube with a diameter of 3 cm. In the bottom of the tube was an opening of 0.5 cm in diameter The glass wool was compressed mechanically for 12 hours. The final length of the glass wool columns used was 3 cm. In the first experiments the glass wool was siliconized before use, but later this was found to be unnecessary Of the freshly drawn heparinized blood 20 ml was immediately poured into the column. Gentle pressure was applied by gas from a tube The gas consisted of 5 % carbon dioxide, 10 % oxygen, and 85 % nitrogen. The pressure was adjusted so as to give a flow rate of about 0 5 ml/min. The operation was performed at 37° C, i e optimal temperature for adhesion of the granulocytes. The first 2 ml of the effluent was discarded, since this fraction contained a considerable number of granulocytes. After the blood had run through the glass wool column, the red blood cells were sedimented by the addition of fibrinogen. The supernatant contained about 70 % of the lymphocytes present in the original blood, but most of the granulocytes and a large fraction of the platelets had been adsorbed to the column. Judging from phase contrast microscopy the cells showed no morphological changes. Electron microscopic examination was performed through the courtesy of Professor G Glimstedt, Department of Histology Lund. No differences were observed between the ultrastructure of the lymphocytes before and after their passage through the glass wool column. In culture more than 10 % of the cells underwent mitosis in the presence of purified phyto-haemagglutinin prepared according to Börjeson (3)

b) *Isolation of the lymphocytes by differential centrifugation.* The method described by Jago (8) was used. The separation of the granulocytes was satisfactory by this technique but the platelet admixture was not eliminated as the platelets remain in the supernatant.

Assay of intracellular glycogen

For the determination of the amount of intracellular glycogen in the different kinds of cell suspensions, the cells were sedimented by centrifugation at 1 000 times gravity (Wifug, rotor diam. 8 cm, 3,500 r p. m.) for 10 min. the plasma was decanted off, and the glycogen in the sediment determined as described previously (4) In order to avoid injury to the cells they were not washed. In a few experiments the decanted plasma was also analyzed for glycogen. Only small amounts were found and residual plasma in the tubes could therefore hardly be of any significance Washing of platelet suspensions 2—3 times with saline decreased their glycogen content by less than 10

Time used for the various stages of the preparation

In all procedures for the fractionation of blood cells, living cells are manipulated for some time with their enzyme systems more or less intact This means that both anabolic and catabolic reactions will proceed after the withdrawal of the blood from the vessel, and that these reactions may alter the glycogen content of the cells. In our experiments the time during which such processes might have occurred was ecorded, i. e. interval between the moment of sampling and addition of sodium hydroxide for determination of the glycogen. This interval was shortest for the platelets, whose isolation took less than 20 min. The isolation of the lymphocytes by differential centrifugation took 30—60 min the preparation of the mixed white blood cell suspensions, about 1 1/2 hours and the isolation of the lymphocytes by glass wool filtration 2—2 1/2 hours.

tion only on the assumption that at least half of the white blood cell glycogen was localized in the lymphocytes (4). Judging from the observations made in the present investigation, however, there is no simple relationship between the glycogen content and the PAS-reaction for the lymphocytes contained only a small percentage of the total glycogen. But these findings do not warrant of any valid conclusions, since we now know that a considerable part of the total glycogen is localized in the platelets, and these cells were not counted in the previous investigation.

The glycogen content per granulocyte now found is less than that reported in our previous paper (4) where the values were not corrected for the platelet content glycogen. It is also less than the value found by Esmann (5, 6) who claimed that his leukocyte suspensions were free from platelets.

Summary

1. *Human* granulocytes, lymphocytes and platelets have been studied for their glycogen content.

2. The platelets were found to have a low glycogen content when calculated per cell but to account for a large part (30—50 %) of the total white blood cell glycogen.

3. The granulocytes were responsible for 50—70 % of the total white blood cell glycogen, while the lymphocytes were poor in glycogen and only accounted for 4—5 % of the total.

4. This implies that the platelet glycogen cannot be neglected in studies of the white blood cell glycogen.

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tively gave 0 8 pg glycogen per lymphocyte and 8.3 pg per granulocyte (fig 1)

Lymphocyte-rich suspensions prepared by differential centrifugation contained 1,500—2,700 lymphocytes, 100—600 granulocytes and 250 000—400 000 platelets per mm³ They were thus much richer in platelets than the preparations obtained after glass wool filtration. After correction for the platelet glycogen the amount of glycogen per leukocyte agreed with the values shown in fig 1

Comparison between the different types of white blood cells

Of the individual cells, the granulocyte proved richest in glycogen with a content of 8.3 pg per cell. The lymphocyte contained 0 8 pg which is only a tenth of that in a granulocyte, and the platelet only 0 092 pg which is about one hundredth of that in a granulocyte. In mixed white blood cell suspensions, however platelets are numerous and therefore contain a considerable fraction of the total glycogen In such suspensions, which had approximately the same relative content of the different types of white blood cells as the whole blood the granulocytes accounted for 50—70 per cent, the platelets for 30—50 per cent, and the lymphocytes for only 4—5 per cent of the total amount of glycogen

Discussion

The most important finding in the present investigation was that a considerable fraction (30—50 %) of the total white blood cell glycogen occurs in the platelets. In previous investigations the amount of glycogen in the platelets has usually been neglected (4 10 13) reference being made to an investigation by Wagner (12) The low glycogen content of the platelets found by Wagner may be explained by the long time used for their isolation. Woodside and Kocholaty (14) have studied the carbohydrate content of human platelets and found the glycogen to amount to 2.34 per cent of the dry weight of the platelets. Since each platelet in their experiments was found to have a dry weight of 2.06 pg, their results indicated a glycogen content per platelet of 0 048 pg Our value, 0 092 pg of glycogen per platelet, is about twice as high Also this difference may be ascribable to the difference in the time used for the isolation of the platelets in the investigation by Woodside and Kocholaty (14) isolation required 6 hours in ours, less than 20 minutes. The glycogen content of the other white blood cells was not studied by Woodside and Kocholaty The bulk of the leukocyte glycogen was found to occur in the granulocytes, a finding in accordance with conclusions drawn by histochemists using the PAS staining technique (11) Leikin s (10) recent assumption that the lymphocyte is the main source of leukocyte glycogen may be explained by the fact that he neglected the amount of glycogen present in the platelets. These cells are very numerous in isolated lymphocyte suspensions obtained by the differential centrifugation method used by Leikin and they account for the major part of the total glycogen. When corrected for the platelet glycogen, we found the glycogen content of the lymphocytes isolated by this method to be no higher than that obtained with the other method used.

In a previous investigation of the relationship between the glycogen content and the periodic-acid-Schiff (PAS) reaction of the leukocytes we found a correla

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Fatal Aplastic Anaemia Following Use of Potassium Perchlorate in Thyrotoxicosis

By

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Antithyroid drugs are often used in the treatment of thyrotoxicosis. Several types of drugs are available, but they may all give rise to undesirable reactions.

Crooks and Wayne (2) in 1960 made a comparison of potassium perchlorate, methylthiouracil and carbimazole in the treatment of thyrotoxicosis in over 450 patients. The incidence of untoward reactions from potassium perchlorate was comparatively low. Untoward reactions were more common with high dosage (1,500—2,000 mg daily) than with low dosage (600—1,000 mg daily). The authors considered potassium perchlorate the drug of choice.

Johnson and Moore (6) reviewed 818 published cases of thyrotoxicosis treated with potassium perchlorate and found 36 cases (4 %) presenting toxic reactions including exanthema, gastro-intestinal disturbances, pyrexia, sore throat, lymphadenopathy and neutropenia. They themselves reported a case of aplastic anaemia.

Hoping to reduce the incidence of toxic reactions from antithyroid therapy Bløstad and Vogt (1) tried a combined treatment with potassium perchlorate and propylthiouracil, using comparatively small amounts of each substance. By the use of 400 mg potassium perchlorate and 400 mg propylthiouracil daily as initial dosage, Vogt (8) reported few untoward effects and satisfactory results in most cases.

Hernberg (4) states that experiences with the use of potassium perchlorate are not exclusively good, but considers the substance a useful alternative in patients who are hypersensitive to other antithyroid compounds.

Four cases of aplastic anaemia due to potassium perchlorate, all of them fatal, have so far appeared in the literature. These patients all received moderate doses, viz., 1,000 mg (6), 800 mg (5, 7) and 600 mg (3) daily.

In an editorial in Brit. med. J. (10) dealing with potassium perchlorate and aplastic anaemia it was pointed out that aplastic anaemia is probably more apt to occur following the use of potassium perchlorate than the organic antithyroid drugs more commonly used during recent years.

Submitted for publication January 15, 1963.

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Book review

Bedside medicine Selected topic. By Erik Ask Upmark. 253 p Sw Crowns 56 — Almquist & Wiksell, Stockholm, Gothenburg Uppsala 1963

An experienced clinician and teacher deals in this book with a subject for which he has a warm corner in his heart. The rapid technical developments in the field of medicine have often resulted in "bedsidemedicine receivingstepmotherly treatment in current textbooks despite its central importance for the description of diseases and practical medical care.

Ask Upmark s book does not claim to be a complete presentation of the subject. It is rather a collection of essays covering different clinical themes. The author's anatomical orientation has resulted in morphology being the basis upon which the presentation usually rests. The amount of factual information which the author has succeeded in presenting in a book of such limited space is impressive, and yet the presentation is clear elegant and inspiriting Case reports from the Medical Clinic in Uppsala and excellent illustrations contribute to the concrete nature of the work. The author's interest in stamps with medical motifs is reflected in the illustrations. For example, the "Local Postal Delivery Centenary stamp is a good illustration of a "marche à petits pas, which was scarcely the intention of the Post Office The book contains a great deal of sound advice. Its tone is both warm and humane.

The book is recommended to the interested student as a complement to current Course literature. It should be a source of enjoyment to every physician irrespective of speciality

The author s profound learning and his powers of observation and combination are abundantly documented. When he occasionally puts forward his own hypotheses, he does so in a severely critical light. His colourful personality and artistic disposition are often manifested in exquisite effects that bring to mind such an author as Karen Blixen.

In this work the author proves himself to be a brilliant clinical teacher

Nils Törnblom

Umeå

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Fatal Aplastic Anaemia Following Use of Potassium Perchlorate in Thyrotoxicosis

By

Nils Gjemdal

Antithyroid drugs are often used in the treatment of thyrotoxicosis. Several types of drugs are available, but they may all give rise to undesirable reactions.

Crooks and Wayne (2) in 1960 made a comparison of potassium perchlorate, methylthiouracil and carbimazole in the treatment of thyrotoxicosis in over 450 patients. The incidence of untoward reactions from potassium perchlorate was comparatively low. Untoward reactions were more common with high dosage (1,500—2,000 mg daily) than with low dosage (600—1,000 mg daily). The authors considered potassium perchlorate the drug of choice.

Johnson and Moore (6) reviewed 818 published cases of thyrotoxicosis treated with potassium perchlorate and found 36 cases (4 %) presenting toxic reactions including exanthema, gastro-intestinal disturbances, pyrexia, sore throat, lymphadenopathy and neutropenia. They themselves reported a case of aplastic anaemia.

Hoping to reduce the incidence of toxic reactions from antithyroid therapy Blomstad and Vogt (1) tried a combined treatment with potassium perchlorate and propylthiouracil, using comparatively small amounts of each substance. By the use of 400 mg potassium perchlorate and 400 mg propylthiouracil daily as initial dosage, Vogt (8) reported few untoward effects and satisfactory results in most cases.

Hernberg (4) states that experiences with the use of potassium perchlorate are not exclusively good, but considers the substance a useful alternative in patients who are hypersensitive to other antithyroid compounds.

Four cases of aplastic anaemia due to potassium perchlorate, all of them fatal, have so far appeared in the literature. These patients all received moderate doses, viz., 1,000 mg (6), 800 mg (5, 7) and 600 mg (3) daily.

In an editorial in Brit. med. J. (10) dealing with potassium perchlorate and aplastic anaemia it was pointed out that aplastic anaemia is probably more apt to occur following the use of potassium perchlorate than the organic antithyroid drugs more commonly used during recent years.

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Case report

A woman, aged 82, was admitted to hospital on Sept. 9 1961 because of malaise, weakness, dyspepsia, loss of appetite and weight, palpitations and loss of hair A diagnosis of thyrotoxicosis was made. Amongst the laboratory findings should be mentioned a white blood count 3,700/mm³ platelet count 225 000/mm Hb 94 % and ESR 8 mm/hr

Treatment with Tapazole® in a dosage of 20 mg daily was started About three weeks later the patient became subfebrile, with pain, swelling redness and restricted movements of the right wrist. Platelets dropped to 154 000/mm³ Tapazole® was withdrawn and treatment with methylthiouracil in a dosage of 300 mg daily was started. Under this treatment marked improvement was noted and the patient was discharged with 200 mg of methylthiouracil daily During the subsequent months the dosage was reduced to 100 mg daily and later to 100 mg every second day

The patient was re-admitted on April 7 1962 because of bronchopneumonia. The white cell count was 3 300/mm³ platelet count 225 000/mm³ The patient received Proca-penicillin. A rash developed, but disappeared following withdrawal of methylthiouracil and Proca penicillin. Later pyuria developed and was treated with a sulphonamide preparation Again, a rash developed, but disappeared following withdrawal of sulphonamide Treatment with potassium perchlorate in a dosage of 600 mg daily was then started Blood values remained normal until July 18, 1962 when the white cell count dropped to 2 600/mm³ Platelets were not counted, but were scanty in a blood smear The haemoglobin content was 84 % The dosage of potassium perchlorate was reduced to 400 mg daily

The patient was admitted again on Aug 15, 1962 because of bleeding manifestations, viz., epistaxis, numerous petecchiae and ecchymoses. Slight sacral and crural oedema was noted. Potassium perchlorate was withdrawn. The urine was normal. The haemoglobin content was 40 % and the red cell count 2 100 000/mm³ The white cell count was 2,100/mm³ with 1 % basophils, 2 % monocytes, 5 % segmented forms and 92 % lymphocytes. Slight anisocytosis and poikilocytosis were noted. The platelet count was 32 000/mm³ ESR 77 mm/hr PP 80 %. A sternal smear showed very few cells, most of them red blood cells, a few mononuclears and few platelets. Megacaryocytes were not seen.

The white cell count ranged between 2,500 and 1,300/mm For a while the platelet count remained at 39 000/mm³ but then dropped to 19 000/mm Otherwise the blood values remained unchanged apart from a transient rise in the percentage of segmented white cells with a corresponding fall in the percentage of lymphocytes, but normal levels were never noted.

The patient was treated with prednisone and a transient reduction of the bleeding manifestations was noted. She also received blood transfusions, courses of Achromycin® and injections of a testosterone preparation, Sustanon® 500 mg weekly Her condition deteriorated and she died on Oct. 20 1962.

Discussion

The criteria for establishing a diagnosis of aplastic anaemia (9) include anaemia, marked granulocytopenia, marked thrombocytopenia and hypocellular bone marrow They were all present in the case reported above.

A rash developed following the administration of sulphonamides, likewise during treatment with methylthiouracil. In the latter event, however the reaction might be due to the concurrent administration of Proca penicillin. Under treatment with Tapazole, arthritis and a fall in the platelet count were noted. In this patient several drugs seemed to cause untoward reactions. As to her aplastic anaemia, however causes other than potassium perchlorate could not be found The initial signs of blood dyscrasia should probably have warranted stricter precautions than merely a reduction of the potassium perchlorate dosage.

Krevans et al. (7) used potassium perchlorate for some time in the treatment of thyrotoxicosis. They found the drug useful in a large proportion of cases, especially in those patients having previously shown sensitivity to propylthiouracil or methimazole or both drugs. These authors, however later observed an instance of fatal aplastic anaemia secondary to the use of potassium perchlorate. As this seems irreversible, the authors later warned against further use of this drug in therapy except under unusual circumstances.

Potassium perchlorate carries a considerable risk, as indicated by the fact that the four published cases of aplastic anaemia following its use all ended fatally. The daily dose in the case reported above was 600 mg which is considered very moderate.

If potassium perchlorate is to be used for treatment of thyrotoxicosis, it should probably not at any rate be given to patients who have shown untoward reactions to any other drug.

Summary

A case of fatal plastic anaemia due to treatment of thyrotoxicosis with potassium perchlorate is reported. The highest dosage level was 600 mg daily. The literature dealing with potassium perchlorate and its complications is briefly reviewed. The patient in question had previously showed untoward reactions to other drugs, and it is probably advisable to avoid the use of potassium perchlorate in patients with previous signs of drug idiosyncrasy.

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Serum Alkaline Phosphatase, SGOT and SGPT During Treatment of Diabetic Patients with Tolbutamide and Insulin

By

R. Keiding and K. Lundbæk

Tolbutamide has now been used extensively for seven years in the treatment of mild or moderate diabetes mellitus in middle-aged and old patients. In general experience the incidence of side-effects has been very low and severe toxic reaction has hardly occurred (5 8).

Clinical signs of *liver damage* has been noted in only a few cases. The jaundice disappeared when the treatment was discontinued (1 7).

Function tests and biopsy studies have usually revealed no untoward influence of tolbutamide treatment on the liver function (2, 3 11).

However Marble and Krall (6) state that a rise in serum alkaline phosphatase occurs in most cases. This rise, which was not accompanied by changes in any other of the commonly used parameters of liver function, was observed after one month and the slightly elevated phosphatase level — about one Bodansky unit higher than before treatment — was still present after about one year' treatment.

This finding might indicate the existence of a mild intracanalicular biliary stasis. Although slight, such an abnormality cannot be entirely neglected if it occurs regularly during the treatment with a very common drug.

The following is report of a study in which serum alkaline phosphatase was followed by determinations at two weeks interval for 6—12 months in a number of diabetic patients who were put on treatment with tolbutamide. To elucidate further the nature of an increase in the level of serum alkaline phosphatase, simultaneous determinations of SGOT and SGPT were carried out in all patients.

Similar investigations were performed on a series of patients put on treatment with insulin.

For the insulin group 16 patients were selected, comparable with respect to age and degree of diabetes mellitus to the patients in the tolbutamide group. The dose of insulin (NPH 50) given was 16—32 units per day.

The serum glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) activity was determined by the method of Reitman and Frankel (9). The range of normal values is SGOT < 40 units and SGPT < 35 units.

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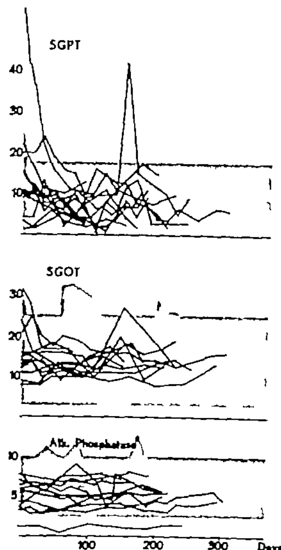


Fig 1 The course of SGPT SGOT and alkaline phosphatase activity in the patients treated with tolbutamide. The shaded area denotes the limits of activity of the enzymes in the group of patients treated with insulin.

Serum alkaline phosphatase activity was determined by the method of Kind and King (4)

The range of normal values is 4—10 K. A. units. In the two series altogether 539 determinations were done for each enzyme.

Results

In fig 1 the course of enzyme levels are depicted The shaded areas show the range of values in the insulin series.

It is seen that there is no pattern of rise or fall from the first pre treatment determination to the first determinations during treatment.

One patient started at a level of 57 units of SGOT This patient was later shown to have cholelithiasis. In another patient SGPT rose to 42 units after 9 months treatment during a course of radium treatment for cancer of the uterus.

During the rest of the period of examination normal values were found. Apart from the two patients mentioned, all the serum enzyme values were found within the normal range, and corresponding to the range of values of the patients treated with insulin.

Discussion

The results of these investigations were entirely negative. It was not possible to confirm the findings of Marble and Krall of a rise in alkaline phosphatase during the treatment with tolbutamide, and no effect was noted on the level of the serum transaminases. The results were exactly the same in patients treated with insulin.

Taken together with earlier published results of other liver function tests and of biopsy studies our results indicated that tolbutamide has no untoward effect on the liver when used in doses of 1—1 1/2 g per day

It is known that during treatment with chlorpropamid another common sulphonamide antidiabetic drug which has the advantage that only one dose a day is required jaundice has occurred in approximately 0.3 % of the patients (10)

Tolbutamide therefore seems to be the sulphonamide compound of choice for the treatment of mild and moderate diabetes mellitus.

Summary

In a group of patients treated with tolbutamide in doses of 1—1 1/2 g per day no significant changes occurred in the level of serum alkaline phosphatase, SGOT and SGPT

The same results were obtained in a comparable group of patients during the treatment with insulin.

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"Thyroglobulin" in the Serum

By

Tage Hjort

Thyroglobulin is a thyroid-specific protein in which active thyroid hormones are incorporated, and from which they can be split off and enter the blood stream. Thyroglobulin is present in the colloid spaces of the thyroid gland, whereas it does not normally occur in the serum in demonstrable amounts (15). However in certain pathological conditions, thyroglobulin may be released from the thyroid gland. By precipitation with an antiserum Lerman (15) was thus able to show that thyroglobulin was often present in blood samples withdrawn from the thyroid veins during operations on the gland. By a 'salting-out' technique combined with ultra-centrifugation, Robbins (17) and Robbins et al. (18) demonstrated circulating thyroglobulin after administration of therapeutic doses of radio-iodine (^{131}I). By means of paper electrophoresis, Stemmermann (24) revealed an increase in the α_2-globulin fraction in four patients with subacute thyroiditis. He interpreted this observation as evidence of the presence of colloid extruded from the thyroid gland. However subsequently Shulman et al. (22) showed that an increased level of α-globulins is a very common occurrence in patients with chronic, non-specific thyroiditis or lymphadenoid goitre. Finally Owen and McConahey (16) demonstrated by means of radio-active iodine a butanol-insoluble, iodine-containing, thyroglobulin-like protein in the serum of the majority of 38 patients with Hashimoto's disease and a few with acute thyroiditis.

After it had been realised that thyroglobulin antibody is frequently present in patients with various thyroid disorders (20, 21) the clarification of the occurrence of thyroglobulin outside the colloid spaces — i.e., under conditions in which it may come into contact with the cells of the immune apparatus — has been thrown into a new perspective. By physical and chemical methods, e.g. by administration of a tracer dose of ^{131}I combined with salting-out and ultra-centrifugation it is possible with quite high certainty to show if it is actually intact thyroglobulin molecules that occur in the serum but these

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Table I The incidence of thyroglobulin antibody (TGA) and "thyroglobulin" in various groups of patients with and without thyroid disease

Clinical diagnoses	No. of cases tested		No. of cases with TGA			No. of cases without TGA		No. of cases with thyroglobulin		
	♂	♀	♂	♀	Total in %	♂	♀	♂	♀	Total in % (of the no. without TGA)
Normal blood donors	97	35	4	0	3	93	35	1	0	1
Patients with various medical diseases, but without thyroid disorders	63	115	2	17	11	61	98	3	8	7
Diffuse non-toxic goitre	13	71	1	6	8	12	65	5	23	36
Nodular non-toxic goitre or adenoma	5	92	1	7	8	4	85	1	20	24
Thyrotoxicosis										
Untreated or treated with iodine only	10	66	3	28	41	7	38	2	10	27
During treatment with Neomercazole	5	29	0	14	41	5	15	3	13	80
Cured for 2 years	5	11	2	2	25	3	9	0	0	0
Toxic adenoma	1	8	0	1	11	1	7	0	3	37
Cancer of thyroid gland	5	12	1	4	29	4	8	2	5	58
Lymphadenoid goitre (Hashimoto's disease)	—	9	—	8	89	—	1	—	0	—
Myxoedema										
Primary untreated	2	6	2	5	63	0	1	0	0	7
Primary treated	—	14	—	9		—	5	—	0	
Postoperative	—	16	—	8		—	8	—	1	
Acute thyroiditis	2	1	1	0	—	1	1	1	0	—

methods are fairly complicated and their sensitivity is at the lower limit of what is needed Immunological demonstration of thyroglobulin is much simpler For example by haemagglutination inhibition it will be easy to obtain the higher sensitivity required (11) It is of secondary importance that the estimation of circulating thyroglobulin by this principle is only semi-quantitative. However a considerable drawback involved in the method is that it responds to all thyroglobulin-antibody fixing groups, i.e. not only thyroglobulin but also any break down products of thyroglobulin in which the determinant (antibody fixing) group is intact. Another weak point in the method is that it permits determination only of free thyroglobulin-antibody-fixing groups so that it cannot be employed if thyroglobulin antibody is present in the serum.

In spite of these methodological disadvantages an attempt was made to assess the occurrence of circulating thyroglobulin by a haemagglutination-inhibition reaction in some patients with or without thyroid disorders.

Table II. The occurrence of thyroglobulin antibody (TGA) and thyroglobulin in 168 patients without thyroid disease

Disease categories	No. of cases tested		No. of cases with TGA		No. of cases without TGA		No. of cases with thyroglobulin	
	♂	♀	♂	♀	♂	♀	♂	♀
Hodgkin's disease	9	8	0	1	9	7	0	0
Multiple myeloma.	2	5	0	0	2	5	0	0
Polycythaemia vera	3	3	0	1	3	2	0	0
Chronic lymphatic leukaemia	1	3	0	1	1	2	0	2
Acute leukaemia	2	1	0	0	2	1	1	1
Lymphosarcoma — reticulosarcoma	4	4	0	0	4	4	0	0
Sarcoidosis	1	—	0	—	1	—	0	—
Myelofibrosis	2	—	0	—	2	—	0	—
Anaemias	2	4	0	0	2	4	0	0
Thrombocytopenia (dis. lupus eryth.?)	—	1	—	0	—	1	—	0
Paraproteinaemia	1	1	0	0	1	1	0	0
Hypersedimentation (unknown cause)	1	—	0	—	1	—	0	—
Subacute hepatocellular disease, cirrhosis	1	2	0	1	1	1	0	0
Rheumatoid arthritis	2	7	0	3	2	4	0	0
Degenerative joint disease	1	10	0	2	1	8	0	1
Cardiovascular disease	7	8	0	3	7	5	0	0
Gastro-intestinal disease	3	4	1	0	2	4	0	1
Carcinoma	2	4	0	1	2	3	0	0
Diabetes mellitus	4	15	1	1	3	14	0	2
Cushing's syndrome	—	1	—	0	—	1	—	1
Simmond's disease	—	1	—	1	—	0	—	0
Exophthalmos	—	1	—	1	—	0	—	0
Miscellaneous.	15	32	0	1	15	31	2	0
Total	63	115	2	17	61	98	3	8
	178		19		159		11	

Material and methods

The series studied consisted of 543 patients, mainly originating from the University Departments of Internal Medicine, Aarhus Kommunehospital, and the Radium Center for Jutland, Aarhus. As far as possible, all patients with overt or suspected thyroid disease were included. The diagnoses were made on the basis of ordinary clinical and laboratory examinations. Determinations of PBI, BMR and serum cholesterol were performed routinely. In addition, the in-vitro uptake of labelled triiodothyronine by the erythrocytes was determined in many patients, and the uptake of ^{131}I by the thyroid gland was measured in selected cases.

Thyroglobulin antibody was demonstrated by Boyden haemagglutination technique (3) as previously described (11). The sheep erythrocytes used had been treated with formalin by the method of Fulthorpe (6) and coated with a purified thyroglobulin fraction prepared by the method of Derrien et al. (5).

Thyroglobulin in the serum was demonstrated by haemagglutination-inhibition test (11) in which the thyroglobulin-antibody fixing activity of the serum was compared with the antibody fixation brought about by known amounts of purified thyroglobulin fraction, so that the thyroglobulin concentrations in the sera could be referred to certain

Table III The age and sex distribution of and the serological findings in 132 healthy blood donors and 178 patients with medical diseases, but without thyroid disorders

	Age (yrs)															
	11–20		21–30		31–40		41–50		51–60		61–70		71–80		Total	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Blood donors																
Total no.	8	3	17	13	29	7	24	6	15	5	4	1	0	0	97	35
No. with TGA	1	0	0	0	1	0	1	0	1	0	0	0	0	0	4	[illegible]
No. with thyroglobulin	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	[illegible]
Medical patients																
Total no.	2	5	10	14	5	16	17	23	18	30	9	22	2	5	63	115
No. with TGA	0	0	0	1	0	2	1	4	0	4	1	5	0	1	2	17
No. with thyroglobulin	0	0	0	0	1	2	0	2	2	1	0	1	0	2	3	8

evels. As the standard thyroglobulin fraction used, which had been prepared from goitrous tissue from a non toxic patient without signs of thyroid auto-immunisation, was not 100 pure the thyroglobulin concentrations revealed in the serum must not be regarded as absolute values but since the same standard was used in all the determinations, the values obtained are comparable

Results

By the technique employed in the present study circulating thyroglobulin can only rarely be demonstrated in normal subjects. In a previous study of 132 healthy blood donors, thyroglobulin was unquestionably demonstrated in the serum in only one case i.e. less than 1 % (11) In order to obtain a further basis for the evaluation of the occurrence of thyroglobulin in individuals without thyroid disease, sera from 178 patients with various medical diseases were studied Thyroglobulin antibody was present in 19 (11 %) of the sera, so that these, for technical reasons, could not be assessed Among the remaining 159 patients, circulating thyroglobulin was present in 11 i e. 7 % (table I)

In table II the 178 patients without thyroid disease proper are listed together with the serological findings. In the previous study of healthy blood donors (12) thyroglobulin antibody was revealed only in 3 % Thus, the antibody occurred with a considerably higher frequency in the group with medical diseases. This is in agreement with the observations of Hackett et al. (7) and the cause of the higher frequency of antibody may as mentioned by Hill (10) be sought partly in the female preponderance and the higher average age of the patients (table III) and partly in the nature of the diseases. Thus, several of the patients with thyroglobulin antibody in the serum had diseases involving the immune apparatus (1 4 9 23) The only patient with exophthalmos without co-existing signs of thyroid disease had also thyroglobulin antibody in a low titre (8)

The medical diseases may partially explain why thyroglobulin also occurred more frequently in the group of patients than in the healthy donors. Thus, three of the four patients with leukaemia who had thyroglobulin in the serum revealed

leukaemic infiltrations of the thyroid gland. One of these showed clinical signs of the infiltrate (swelling and tenderness of one thyroid lobe) while the infiltrations were confirmed by histological examination in the other two. One of the latter — a 31-year-old woman with blast leukaemia in whom autopsy subsequently revealed diffuse infiltration of the thyroid tissue by blastlike cells — had 0.5 μg thyroglobulin per ml serum. At the same time, the serum yielded by the application of Coons' sandwich technique to methanol-fixed sections of thyroid tissue, the bright uniform fluorescence of the colloid spaces which, according to Balfour et al. (2) is characteristic of antibody against the second colloid antigen (CA-2-antibody). Simultaneous occurrence of free, thyroglobulin-antibody-fixing groups and CA 2-antibody in the serum was also observed in several patients with thyroid disorders. This observation provides strong evidence in support of the assumption that the two antigen-antibody systems are of an entirely different nature.

The patient with Cushing's syndrome had for two years been treated for a tuberculous renal disease, and the treatment with para-aminosalicylic acid (PAS) had been discontinued only three months before the test was performed. Although goitre could not be demonstrated, it can scarcely be excluded that the prolonged PAS therapy may have been a contributory cause of the presence of thyroglobulin in the serum. The group "Miscellaneous" mainly comprised patients with mild psychic disorders. One of the two women with circulating thyroglobulin suffered from severe obesity and her own doctor had for four years treated her with desiccated thyroid. On admission to the medical department, desiccated thyroid was gradually withdrawn, and the serum sample was secured during this period. Thus, in five of the eleven cases, a possible explanation could be found for the presence of thyroglobulin in the blood stream, while no such explanation could be offered in six of the patients without actual thyroid disease.

The conditions observed among the patients with thyroid disorders were highly different (table I). Thyroglobulin antibody was here revealed only in 8 % of 84 patients with diffuse non-toxic goitre and also in 8 % of 97 patients with nodular non-toxic goitre. On the other hand, circulating thyroglobulin was frequently found among the patients without thyroglobulin antibody in the same two groups, viz. in 36 and 24 % respectively. From fig. 1 it appears that the thyroglobulin concentrations disclosed were relatively low. Owing to the difficulties involved in an objective evaluation of the size of a goitre, no attempts were made to relate the frequency of circulating thyroglobulin to the size of the goitre. However, no such relationship seemed to exist, since thyroglobulin was revealed in several patients with very small goitres. Among eight patients with intrathoracic goitre (nodular 1, diffuse 7) thyroglobulin was present in the serum of four. One of these — an 85-year-old man with a large, firm, partially intrathoracic goitre — occupied a particular position. He was the only one in the group who had a serum thyroglobulin concentration of about 10 μg/ml, and both complement-fixing antibody and CA 2-antibody were present in the serum. In this patient, the goitre was only a secondary finding, and in view of his advanced age and poor general condition no further investigation into the nature of the goitre was performed as it did not cause any serious discomfort.

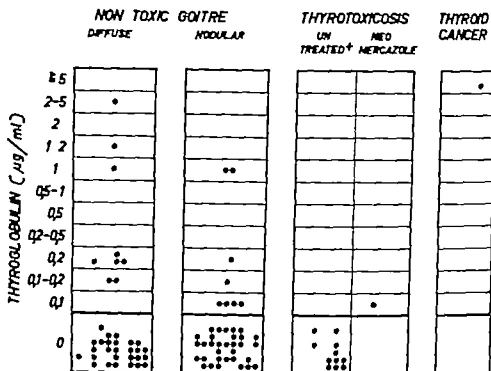


Fig 1 The thyroglobulin concentrations revealed in patients with non-toxic goitre, thyrotoxicosis and thyroid cancer
○ Males. ● Females.
+ Untreated or treated with iodine only

In 31 (41 %) of 76 patients with thyrotoxicosis who were either untreated or had been given iodine treatment only the sera contained thyroglobulin antibody so that tests for thyroglobulin could be performed only in 45 cases. In 12 of these (27 %) thyroglobulin was present, in most cases in relatively low concentrations (fig 1) Although thyroglobulin antibody was present in this group about five times as frequently as in patients with non toxic goitre thyroglobulin was thus disclosed with roughly the same frequency and in the same concentrations among the patients without antibody in the two groups. On the other hand during treatment with Neomercazole (2-carbethoxy thiamazole) 16 (80 %) of the 20 patients without thyroglobulin antibody revealed circulating thyroglobulin. Ten of these had been tested before the commencement of therapy As thyroglobulin was found in only 2 of these 10 cases at that moment, and as the thyroglobulin disappeared again after the discontinuance of treatment in 7 of 8 cases followed it is reasonable to assume that the presence of thyroglobulin in the sera of these patients was connected with Neomercazole therapy In fig 2 (upper part) the thyroglobulin findings in some typical cases are related to the duration of the treatment. None of the patients showed appreciable growth of the thyroid gland during treatment.

If the antigenic properties of the circulating thyroglobulin-antibody fixing components are preserved the possibility exists that Neomercazole treatment may result in the development of an auto-immunisation in patients who are not immune-tolerant to thyroglobulin. Only in two patients did thyroglobulin anti-

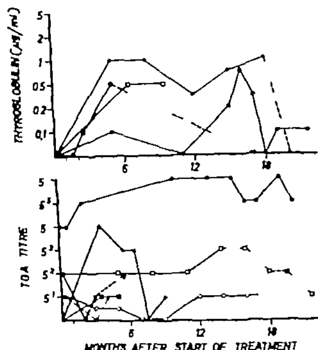


Fig. 2. The fluctuations in thyroglobulin- and thyroglobulin antibody concentrations during treatment with Neomercazole.
—— Under treatment.
After treatment.

body develop during the first months of treatment with Neomercazole (fig. 2, lower part). In patients with thyroglobulin antibody in the serum, leakage of thyroglobulin-antibody fixing groups from the thyroid gland may be supposed to cause a partial or complete neutralization of the antibody. As might be expected, the thyroglobulin-antibody titres in the patients treated with Neomercazole also showed some fluctuations, and in four cases the antibody disappeared temporarily but free thyroglobulin could not be demonstrated in the serum (fig. 2, lower part). Mild cases of auto-immunization against thyroglobulin may thus — at least temporarily — be masked during treatment with Neomercazole.

The occurrence of thyroglobulin antibody and thyroglobulin in 23 thyrotoxic ... and in 30 without eye symptoms is shown in table IV. Only patients for whom unquestionable and concordant information was available were included, and gradation of the severity of the exophthalmos was not attempted. Assessed on this basis, the observation reported by Hales et al. (8) viz. that thyroglobulin antibody occurs with a very high frequency in patients with exophthalmos, could not be confirmed. The two groups did not show any difference as regards the serological findings.

The group with cancer of the thyroid gland consisted of 15 cases of histologically confirmed carcinoma (including four of anaplastic carcinoma) and two in which histological examination showed no signs of malignancy but revealed ectopic thyroid tissue in the cervical lymph nodes. Five of these patients revealed thyroglob-

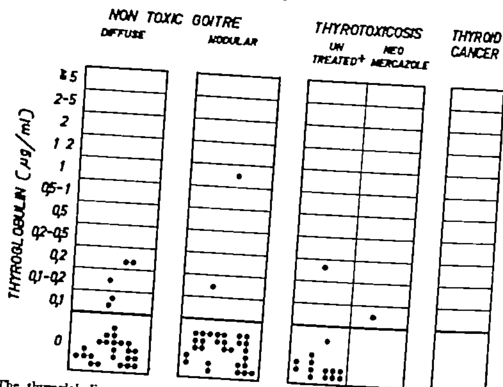


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If the antigenic properties of the circulating thyroglobulin antibody fixing components are preserved the possibility exists that Neomercazole treatment may result in the development of an auto-immunisation in patients who are not immune tolerant to thyroglobulin. Only in two patients did thyroglobulin anti-

ment of auto-immunisation with formation of thyroglobulin antibody. However as only a few of the patients considered here responded in this way, it must be assumed that the majority of the patients were immune-tolerant to thyroglobulin. Further evidence in support of this assumption is provided by the fact that thyroglobulin-antibody-fixing components can be demonstrated in cord blood from most newborn infants (13).

While thyroglobulin antibody was five times as frequent in patients with thyrotoxicosis as in patients with non-toxic goitre, circulating "thyroglobulin" occurred roughly with the same frequency and in the same concentrations among the patients in the two groups who had no antibody in the serum. If the explanation of the development of the thyroglobulin auto-immunisation is to be sought exclusively in the absence of immune-tolerance in the individual patient ("disturbed-antigen" disease) it might be concluded that thyrotoxicosis would chiefly occur in persons who were not immune-tolerant to thyroglobulin. As adequate evidence is not available in support of such a view, which would imply that this form of auto-immunisation would be the existing factor in the development of thyrotoxicosis in some of the patients, the explanation of the auto-immunisation must also be sought in other factors.

It is a well-known fact that patients with severe forms of thyroid auto-immunisation (as in Hashimoto's disease) often have other co-existing diseases in which auto-immune reactions are believed to play a part, e.g. lupus erythematosus or rheumatoid arthritis (4, 9, 25) and it has also been shown that thyroid antibodies are often present in diseases associated with hypergammaglobulinaemia (23). In these cases the development of auto-immunisation may partially be ascribed to an abnormal immune apparatus ("disturbed-tolerance" disease) but in thyrotoxicosis such an explanation does not seem satisfactory.

A third possibility must therefore be considered, viz. a change in the structure of the thyroglobulin molecule. If thyrotoxic patients give off a changed thyroglobulin molecule (or parts of it) with antigenic properties which are stronger than those of the normal thyroglobulin molecule (e.g., an incompletely folded molecule with an increased number of free antigenic groups), this would offer an explanation for the difference in frequency with which thyroglobulin antibody occurs in thyrotoxicosis and in non-toxic goitre.

On the basis of the results of the present study no conclusions can be drawn as to a possible change in the structure of thyroglobulin in thyrotoxic patients, but since studies on the thyroglobulin pool of the thyroid gland (14) may be interpreted in the same way, it seems reasonable to point to the possibility.

Summary

The content of thyroglobulin-antibody fixing components ("thyroglobulin") was determined in the serum of patients without thyroglobulin antibody by means of a haemagglutination-inhibition test.

Among 139 medical patients without thyroid disease "thyroglobulin" was revealed in 11 (7 %), whereas among the patients with non-toxic goitre or thyrotoxicosis a positive result of the haemagglutination-inhibition test was obtained in between one-third and one quarter of the cases studied. In thyrotoxic patients under treatment with Neomercazole, thyroglobulin antibody-

Table IV The occurrence of thyroglobulin antibody (TGA) and thyroglobulin in 53 thyrotoxic patients related to the presence of exophthalmos

	No. of pat.	No. with TGA	No. with thyroglobulin	Neither TGA nor thyroglobulin
Exophthalmos	23	9	3	11
No exophthalmos	30	10	6	14

ulin antibody in fairly low titres, and thyroglobulin was demonstrated in seven. In three of 12 patients with unquestionable metastases and the two with aberrant thyroid tissue the serum thyroglobulin concentrations were exceptionally high (5 μg/ml or more) Outside the group with cancer of the thyroid an equally high thyroglobulin concentration was found only in one patient, viz the aforementioned 85-year-old man with a large, firm diffuse non-toxic goitre in whom both CA 2-antibody and complement fixing antibody were demonstrated. The high thyroglobulin concentrations observed in the present study are in good agreement with the results of chromatographic studies reported by Robbins et al. (19) who found signs of the presence of circulating thyroglobulin in six of 23 patients with carcinoma of the thyroid gland.

A man aged 22, with an adenocarcinoma of the thyroid gland and metastases to the cervical lymph nodes revealed 0.5 μg thyroglobulin per ml serum. He was subjected to total thyroidectomy with removal of the lymph nodes in the lateral cervical region. Three weeks after the operation, the thyroglobulin concentration in the serum was unchanged for which reason it must be regarded as doubtful whether all metastases had been removed

The evaluation of the occurrence of thyroglobulin in patients with lymphadenoid goitre or myxoedema is difficult because most of these patients have thyroglobulin antibody in the serum. Among the 14 patients with primary or postoperative myxoedema in whom no antibody was present, thyroglobulin was demonstrated only in one patient with postoperative myxoedema. (Several tests performed in the observation period before therapy with desiccated thyroid was instituted showed concentrations ranging from 0 1 to 0.2 μg/ml.)

One of the three patients with acute thyroiditis had in the initial test performed at a time when swelling and tenderness of the thyroid gland were still present, 0 1 μg thyroglobulin per ml serum (and both CA 2 antibody and complement fixing antibody were simultaneously demonstrated) Small amounts of thyroglobulin antibody were demonstrated a fortnight later while the antibody had disappeared when a repeat test was performed after the lapse of four months. As the patient had previously for a couple of months had mild toxic symptoms and a small goitre, the reaction might possibly be interpreted as an acute flare-up of an autoimmune thyroiditis.

Discussion

The presence of thyroglobulin or its incomplete breakdown products in the serum must be taken as a manifestation of a leakage from the colloid follicles in which thyroglobulin is normally found. In individuals who are not immunetolerant to thyroglobulin, such a constant leakage of colloid components might provide good conditions for the develop-

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The Occurrence of Antibody Against "Second Colloid Antigen" (CA-2 Antibody) in Patients with and without Thyroid Disease

By

Tage Hjort

For several years, Coons' "fluorescent antibody" technique has been used in the clarification of the histological localisation of thyroid auto-antibodies. White (23) found that thyroid antibody was bound to the colloid and the follicular epithelium of the thyroid gland, and this observation was confirmed by Hiramoto et al. (15). In rabbits which had been actively immunised with autologous thyroid extract, the thyroid auto-antibodies were also localised to the colloid (2). Landing et al. (19) demonstrated thyroid antibodies which stained the cytoplasm of the glandular epithelium without concurrently staining the colloid, and Holborow et al. (17) showed that it is only the "complement-fixing thyroid antibody" that stains the cytoplasm of the glandular cells, while the colloid is stained by thyroglobulin antibody. In 1959 White (24) detected a third antibody which stained the cell nuclei, but this antibody was not thyroid-specific. The comprehensive immunofluorescent studies performed by Beutner and Witebsky (3) confirmed the presence of these three different antibodies, each with its own characteristic histological localisation.

Crawford et al. (9) and Lessof et al. (20) used a fluorescent-spot technique for routine demonstration of thyroglobulin antibody, but otherwise the tanned-cell technique is usually preferred for this purpose because of its higher sensitivity. On the other hand, for the demonstration of the complement fixing antibody, Beutner and Witebsky (3) recommended Coons' technique in preference to the complement-fixation test, because of its higher specificity. However, if the specificity of the complement fixation reaction is checked by concurrent studies of the response of the serum to antigens other than thyroid extract (e.g. liver extract), the two methods seem to give roughly the same results.

Blizzard et al. (4) and Doniach et al. (11) observed staining of the colloid by sera which gave a negative haemaggluti-

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fixing groups were revealed in the serum of four fifths of the cases, and in patients with small amounts of thyroglobulin antibody the leakage of such components from the thyroid gland sometimes resulted in partial or complete neutralisation of the antibody

The above-mentioned groups of patients revealed only small amounts of circulating "thyroglobulin usually less than 1 μg per ml serum However in five of 12 patients with carcinoma of the thyroid gland or ectopic thyroid tissue, considerably higher concentrations were demonstrated viz 5 μg/ml or more.

Since then, a leakage of the colloid follicles apparently occurs fairly frequently in patients with thyroid disorders it is discussed why only some of the patients — and a varying fraction of the patients in the various disease groups — produce thyroglobulin antibody

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Table I. Antibody findings in 180 patients with medical diseases, but without actual thyroid disorders

Disease categories	No. of cases tested		No. of cases with antibody											
			CA 2 A only		TGA only		CFA only		CA-2 A + CFA		TGA + CFA	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Hodgkin's disease	8	8	0	0	0	0	0	0	0	0	0	1
Multiple myeloma	2	5	1	0	0	0	0	0	0	0	0	0
Polycythaemia vera.	3	5	0	0	0	1	0	0	0	0	0	0
Chronic lymphatic leukaemia	1	5	0	0	0	1	0	0	0	0	0	0
Acute leukaemia	3	1	0	1	0	0	0	0	0	0	0	0
Lymphosarcoma — reticulosarcoma	4	4	0	0	0	0	0	0	0	0	0	0
Sarcoidosis	1	—	0	—	0	—	0	—	0	—	0	—
Myelofibrosis	2	—	0	—	0	—	0	—	0	—	0	—
Anaemia	2	4	0	0	0	0	0	0	0	0	0	0
Thrombocytopenia (dum. hypos eryth?)	—	1	—	0	—	0	—	1	—	0	—	0
Paraproteinaemia	1	1	0	0	0	0	0	1	0	0	0	0
Hypersedimentation (unknown cause)	1	—	0	—	0	—	0	—	0	—	0	—
Subacute hepatocellular disease, cirrhosis	1	2	0	0	0	0	0	0	0	0	0	1(1)
Rheumatoid arthritis	2	7	0	0	0	2	0	0	0	0	0	1
Degenerative joint disease	1	10	0	0	0	2	0	0	0	0	0	0
Cardiovascular diseases	7	8	0	0	0	2(1)	0	0	0	0	0	1
Gastro-intestinal diseases	3	4	0	0	1	0	0	0	0	0	0	0
Carcinoma	2	4	0	0	0	1	0	0	0	0	0	0
Diabetes mellitus	4	15	0	0	1	1	0	0	0	0	0	0
Cushing's syndrome	—	1	—	0	—	0	—	0	—	0	—	0
Simmonds' disease	—	1	—	0	—	1	—	0	—	0	—	0
Exophthalmos	—	1	—	0	—	1	—	0	—	0	—	0
Miscellaneous	16	33	0	0	0	0	0	0	0	0	0	1(1)
Total	64	116	1	1	2	12	0	2	0	0	0	5
	180		2		14		2		0		5	

CA-2-A = CA-2 antibody; TGA = Thyroglobulin antibody; CFA = Complement-fixing antibody

Non-thyroid-specific complement-fixing antibody

The figures in parentheses indicate the number of cases in which CA-2 antibody occurred simultaneously with thyroglobulin antibody

et al. occurred. Only reactions in which all the colloid spaces of the section concerned were stained were recorded as positive.

Thyroglobulin antibody was demonstrated by Boyden haemagglutination technique (3) with formalin-treated, thyroglobulin-coated sheep erythrocytes.

Complement-fixing antibody was revealed by Donnelley semi-micro complement-fixation technique (10), exactly as described by Roitt and Doniach (21). The specificity of the antibodies was checked by simultaneous observation of the reaction of the serum with liver extract.

nation reaction with thyroglobulin-coated cells, and from the results obtained by Balfour et al. (1) it became evident that a third thyroid-specific antibody — CA 2 antibody — may also occur in the serum. Since, so far only by Coons technique has it been possible to reveal the presence of CA 2 antibody this method has now become indispensable in the complete clarification of the phenomena of auto-immunisation in patients with thyroid disorders. The CA 2 antibody reacts with an antigen in the colloid — the second colloid antigen (CA 2) — the nature of which is as yet unknown In the presence of CA 2 antibody Coons sandwich technique yields a uniform, bright fluorescence of the colloid spaces Generally this reaction can readily be distinguished from the floccular appearance of the colloid which occurs when thyroglobulin antibody is present in the serum.

Even in the sandwich version Coons technique is fairly complicated, requiring as it does, much time and a considerable technical equipment. In deciding whether or not there is any reason to use Coons technique together with the tanned-cell and complement fixation reactions in serological routine studies of patients with thyroid disease, it must be considered how often thyroid auto-immunisation can be revealed only by Coons method In the groups of patients studied both Balfour et al. (1) and Chandler et al. (7) found CA 2 antibody with roughly the same frequency as thyroglobulin antibody but some of the patients with CA 2 antibody also revealed complement fixing antibody or thyroglobulin antibody so that the presence of an auto-immunisation process could be recognised by the routine methods alone.

In an attempt to assess the value of Coons technique as a routine method for the demonstration of CA 2 antibody, the occurrence of the three thyroid-specific antibodies was studied in patients with various thyroid disorders and in a group without such disorders.

Material and methods

With a few exceptions, viz. the cases in which the amount of serum available was insufficient for all the determinations, this investigation comprised the same patients as were studied for "thyroglobulin" (15) The selection of the patients and the diagnostic considerations have thus previously been mentioned. Sera from a total of 550 patients were studied for the presence of the three thyroid-specific antibodies.

CA 2 antibody was demonstrated by Coons sandwich technique on methanol-fixed frozen sections of thyroid tissue as described by Balfour et al. (1) Thyroid tissue removed at operation was cut into cubes of a suitable size and frozen as quickly as possible by solid CO_2. Stored under solid CO_2, such cubes could be used for about four weeks. Sections, about 6 μ in thickness, were cut in a Pearse cryostat. After drying in air they were fixed in absolute methanol for 1 hour The staining procedure was exactly as described by Balfour et al. Commercially available fluorescein-labelled anti-human globulin (Baltimore Biological Laboratory Inc.) was used, absorbed as required with acetone-dried mouse-liver powder (8) in order to eliminate non-specific staining The fluorescent microscopy was performed by means of Leitz fluorescent equipment. Duplicate determinations were performed on all sera studied, and in each series a serum giving a positive reaction of medium strength was included as a control.

As thyroglobulin antibody also stains the colloid by Coons technique, only sera which did not contain thyroglobulin antibody or in which it was present in so minute amounts that it could not give any definite staining of the colloid (haemagglutination titres below 100) were studied. In the presence of CA 2 antibody the bright green uniform fluorescence of the colloid described by Balfour

for which reason it must be assumed that these sera also contained CA 2 antibody

While CA 2 antibody was thus demonstrated in at most five of the patients without thyroid disease, thyroglobulin antibody was present in 19 However this does not necessarily imply that CA 2 antibody actually occurs so much more rarely than thyroglobulin antibody but should rather be taken as signifying that the sensitivity of Coons technique is lower than that of the tanned-cell technique.

The frequency of the antibodies in the various groups of patients is shown in table II. Eight (8 %) of the 98 patients with diffuse, non-toxic goitre and nine (9 %) of the 98 with nodular non-toxic goitre had only CA-2 antibody in the serum. Thus, nearly one-half of the demonstrated cases of auto-immunisation in these groups could be disclosed only by Coons technique. In one patient with diffuse, non toxic goitre both CA-2 antibody and complement fixing antibody were present. Clinically it may be difficult to decide whether a given patient suffers from simple diffuse, non-toxic goitre, or if nodular changes in the thyroid gland are present. Such difficulties involved in the classification may have been a contributory cause of the almost identical serological findings in the two groups in each of the groups, auto-immunisation was demonstrated in about one fifth of the patients studied.

In the serum of one of the patients with nodular goitre, Coons reaction yielded bright, uniform staining of the colloid i.e. a typical CA 2 response. However at the same time the haemagglutination test gave an atypical reaction, viz. a distinct zone phenomenon, so that the reaction was negative in the serum dilutions 1 5 and 1 25 and became positive only in the dilutions 1 125 1 625 and 1 3 125 As the reactions were negative in the low serum dilutions, the samples from this patient could be studied for thyroglobulin by the haemagglutination inhibition technique (14) Immediately after partial thyroidectomy a transient occurrence of thyroglobulin could be demonstrated in the serum, while the atypical haemagglutination reaction remained unchanged. Accordingly the possibility of the presence of an atypical thyroglobulin antibody thus seems to be ruled out. It is reasonable to assume that the serum of this patient contained an uncharacteristic rare colloid antibody (cf. Balfour et al. (1))

Among the 99 patients with thyrotoxicosis studied the sera revealed thyroglobulin antibody in 39 complement-fixing antibody in 32 and CA-2 antibody in 21 In these cases, CA-2 antibody thus showed the lowest frequency and only in nine (9 %) was it found alone so that the presence of auto-immunisation could be recognised only by Coons technique. Four of the nine patients with toxic adenoma revealed antibodies including two in whom only CA-2 antibody was present.

The group with cancer of the thyroid comprised 14 patients with histologically confirmed carcinoma (including four with anaplastic carcinoma) and two who had ectopic thyroid tissue in the cervical lymph nodes, but in whom the histological examination had failed to reveal signs of unquestionable malignancy In this group CA 2 antibody showed the highest incidence of occurrence as five of the patients had this antibody in the serum, including three in whom it was the only antibody present. Four had thyroglobulin antibody in low titres, and four had complement fixing antibody Auto-immunisa-

Table II Antibody findings in the 550 patients studied

Clinical diagnoses	Sex	No. of cases tested	Serological findings											
			CA-2 A only		TGA only		CFA only		CA 2 A + CFA		TGA + CFA		No anti-bodies	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Patients with various medical diseases, but without thyroid disorders	♂	64	1		2		0		0		0		61	
	♀	116	1	1	12(1)	8	2	1	0	0	5(2)	3	96	87
Diffuse non-toxic goitre	♂	15	1	7	1	7	0	0	1	7	0	0	12	80
	♀	83	7	8	6(1)	7	1	1	0	0	1	1	68	[illegible]
Nodular non-toxic goitre or adenoma	♂	5	0	0	0	0	0	0	0	0	1	20	4	80
	♀	93	9	10	6(1)	6	2	2	0	0	1	1	75	81
Thyrotoxicosis	♂	12	1	8	1	8	2	17	1	8	1(1)	8	6	50
	♀	87	8	9	19(1)	22	4	5	6	7	18(3)	21	32	37
Toxic adenoma	♂	1	1	—	0	—	0	—	0	—	0	—	0	—
	♀	8	1	12	0	0	1	12	0	0	1	12	5	63
Cancer of thyroid gland	♂	5	1	20	0	0	0	0	1	20	1	20	[illegible]	40
	♀	11	2	18	2	18	0	0	1	9	1	9	5	45
Lymphadenoid goitre (Hashimoto disease)	♂	0	—	—	—	—	—	—	—	—	—	—	—	—
	♀	9	0	0	0	0	0	0	1	11	8(1)	88	0	0
Myxoedema														
Primary untreated	♂	2	0	0	0	0	0	0	0	0	2	100	0	0
	♀	6	0	0	3(1)	50	0	0	1	17	2	33	0	0
Primary treated	♂	0	—	—	—	—	—	—	—	—	—	—	—	—
	♀	14	2	14	8(2)	57	0	0	0	0	1	7	3	21
Postoperative	♂	0	—	—	—	—	—	—	—	—	—	—	—	—
	♀	16	0	0	5(1)	31	1	6	2	12	3(1)	19	5	31
Acute thyroiditis	♂	2	0	—	1(1)	—	0	—	1	—	0	—	0	—
	♀	1	1	—	0	—	0	—	0	—	0	—	0	—

1 atypical CF A not thyroid-specific; later also TGA.

Symbols as in table I. The figures in parentheses indicate the number of cases in which CA 2 antibody occurred simultaneously with thyroglobulin antibody

Results

The occurrence of the various thyroid antibodies in patients with medical diseases but without actual thyroid disorders is listed in table I. Among the 180 patients studied CA 2 antibody alone was present in the sera of only two (i.e. 1 %) In one of these a female patient with acute leukaemia subsequent autopsy revealed diffuse infiltration of the thyroid tissue by blast-like cells In three cases in which the sera contained small amounts of thyroglobulin antibody (haemagglutination titres ≤ 25) Coons technique yielded bright uniform staining of the colloid

thyroid diseases, although it is not found quite so often as the other two thyroid-specific antibodies. Since, like complement-fixing antibody CA-2 antibody can only rarely be revealed in patients without thyroid disease, the demonstration of the presence of these antibodies may be of value in clinically doubtful cases of thyroid disorders.

Thyroid serology has become of some importance in choosing treatment in goitrous patients, because several studies have shown that postoperative myxoedema often develops in patients who at the time of operation, have complement fixing antibody or a high concentration of thyroglobulin antibody in the serum (6 12, 16 18) However the significance of CA-2 antibody in the development of postoperative myxoedema had not yet been studied.

Balfour et al. (1) who studied sera which gave a haemagglutination titre ≦ 250 found that CA-2 antibody was present in 41 % of 122 patients with thyrotoxicosis and in 16 % of 68 with non-toxic, nodular goitre. In their series originating from England, CA 2 antibody was thus present in a somewhat higher percentage than in the groups of patients considered here. Technical differences may play a part, but as thyroglobulin antibody and complement-fixing antibody apparently also occur more frequently among English patients than among those of the present study (cf. Roitt & Doniach 1960 (22)) it may be reasonable to consider the influence of geographical factors.

Summary

Coons sandwich technique was used in the study of the occurrence of CA antibody (antibody to the second colloid antigen) in 180 patients with medical diseases but without actual thyroid disease and in 370 patients with various thyroid disorders.

Among the patients without thyroid disease CA 2 antibody was present in 3 %, but only in 1 % was it found alone

On the other hand CA 2 antibody occurred fairly frequently among patients with thyroid disorders, often in combination with one, or both of the other two thyroid antibodies. However in 9 % of 196 patients with non-toxic goitre and in 9 % of 99 patients with thyrotoxicosis CA-2 antibody occurred alone, so that only Coons technique could reveal the presence of auto-immunisation.

In patients with lymphadenoid goitre or myxoedema, Coons technique was of less value, since most of these patients had thyroglobulin antibody in the serum in such high titres that CA 2 antibody if any for technical reasons could not be demonstrated.

All of three patients with acute thyroiditis had CA-2 antibody in the serum.

In six patients with CA-2 antibody who underwent partial thyroidectomy leakage of the second colloid antigen could not be demonstrated indirectly (i.e. by a transient fall in the amount of circulating CA 2 antibody) during the immediate postoperative period

Acknowledgements

I am indebted to Dr R. Kelding, M.D Head of the Central Laboratory Aarhus Kommunehospital, for the rotary serum samples I have received from his department, and to Professor K. Lundbæk, M D for his permission to study patients from the Second Medical University Department, Aarhus Kommunehospital, for the purpose of this investigation.

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Table III Concentrations of CA 2 antibody just before and after partial thyroidectomy

Clinical diagnoses	Sex	Age (yrs)	CA 2 antibody titre			
			Before op	Postoperative findings		
				1st day	3rd day	6th day
Thyrotoxicosis	♀	16	60	60	60	40
Thyrotoxicosis	♀	28	5	5	2	2
Thyrotoxicosis	♀	34	5	5	5	5
Thyrotoxicosis	♀	38	2	2	2	2
Nodular non-toxic goitre	♀	33	1	1	1	1
Nodular non-toxic goitre	♀	55	1	1	1	1

A titre of 1 indicates that only undiluted serum gave an unquestionably positive Coons reaction.

tion was thus demonstrated in nine of these 16 patients.

Most of the patients with lymphadenoid goitre or myxoedema had thyroglobulin antibody in high concentrations As absorption of thyroglobulin antibody was not attempted it could not be ascertained whether or not CA 2 antibody was present in these cases. Only in two patients who were under treatment for myxodema was CA 2 antibody revealed alone in four myxoedematous patients it was found together with small amounts of thyroglobulin antibody and in one patient all three antibodies were present. One or more of the antibodies could be demonstrated in all the patients with lymphadenoid goitre or untreated myxoedema.

Acute thyroiditis occurred in only three patients. All three revealed CA 2 antibody in the serum in one alone, in one together with a small amount of thyroglobulin antibody and in the third in combination with complement fixing antibody During the acute phase of the disease the serum of the last of these patients contained thyroglobulin but later thyroglobulin antibody was present in a low titre.

After operations on the thyroid gland leakage of thyroglobulin may occur resulting in a transient complete or partial neutralisation of thyroglobulin antibody if this is present (14) In order to assess if similar conditions prevail as regards the CA 2 system, the serum concentration of CA 2 antibody was determined just before and on the first, third and sixth days after partial thyroidectomy in six patients in whom this antibody had previously been revealed. The amounts of antibody present were expressed by the highest serum dilution which still gave an unquestionable uniform staining of the colloid by Coons technique. All samples from the same patient were studied simultaneously The results are shown in table III and it appears that only in one of the six patients did a fall in the serum concentration of CA-2 antibody seem to occur on the third postoperative day However the lack of indirect demonstration of postoperative leakage of the second colloid antigen here may be due to the relatively low sensitivity of Coons technique.

Discussion

The results reported here confirm that CA 2 antibody can be demonstrated fairly frequently in patients with various

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By

Bengt Odeberg, Harald A. Hansen and Lars Olof Lanner

Megaloblastic anemia of the type seen in some fish tapeworm (*Diphyllobothrium latum*) carriers is generally ascribed to vitamin B_{12} deficiency (1–4). Thus Palva (17) found that a high proportion of a large series of tapeworm carriers showed a reduced serum vitamin B_{12} level and an abnormal Schilling test. These findings were usually well correlated with the morphological bone marrow picture. Notably however 21 of 82 subjects (26 %) who exhibited evidence of antipernicious anemia factor deficiency in the bone marrow — megaloblastic features and/or over 30 per cent giant metamyelocytes — had normal serum levels of vitamin B_{12}. This suggests that vitamin B_{12} deficiency might not be the sole or only cause of megaloblastic bothriocephalus anemia. The most likely other causative factor would be folic acid deficiency. The folic acid metabolism of fish tapeworm carriers is imperfectly understood. There is some evidence that these subjects may have folic acid deficiency and that it could be a factor in the causation of their megaloblastic anemia. Studying the urinary excretion of folic acid and vitamin B_{12} in fish tapeworm carriers with and without megaloblastic anemia, Markkanen et al. (14) observed that the excretion of folic acid and vitamin B_{12} in the anemics was even lower than the significantly subnormal excretion in the non anemics. The mean folic acid excretion differences between groups were of the same order as the corresponding vitamin B_{12} differences. And Gräsbeck et al. (5) found an abnormal formiminoglutamic acid (FIGlu) excretion in one of 9 fish tapeworm carriers.

In cases of gross *Anchylostoma duodenale* infestation with grave iron deficiency anemia patients exhibit low serum vitamin B_{12}, defective folic acid absorption and signs of folic acid deficiency (low folic acid clearance) but their erythropoiesis lacks megaloblastic features (13)

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In the case of megaloblastic anemia in a tapeworm carrier to be reported here, it could be demonstrated that folic acid deficiency was a major factor in the causation of the anemia. The possibility of using "diagnostic folic acid doses for differentiating various types of megaloblastic bothriocephalus anemia will be discussed in the light of this case.

Methods

Serum vitamin B_{12} was assayed microbiologically according to Hutner Bach and Ross (12) using the Z strain of *Euglena gracilis* The serum vitamin B_{12} level of healthy controls ranges from 175 to 960 pg/ml and averages 379 pg/ml

Serum and whole blood folic acid levels were determined as described previously (6 16) The serum folic acid level in healthy controls ranges from 3.2 to 13 8 mμg/ml, the median being 5 4 mμg/ml. Values below 2.5 mμg/ml usually indicate folic acid deficiency However values between 2.5 and 3.2 mμg/ml are seen quite often in cases without clinical manifestations of folic acid deficiency

Folic acid absorption was assessed with the aid of a slightly modified (16) form of the method described by Chanarin et al (3)

Urinary FIGlu excretion after histidine loading was estimated according to Tabor and Wyngarden (19) in the manner recommended elsewhere (7 8) Thus determined, the urinary FIGlu output of healthy subjects averages 96 μM in 8 hours (12 μM/h) Concentrations exceeding 220 μM over 8 hours (27 μM/h) are abnormal.

Therapy with small diagnostic" doses of folic acid (0.2 mg daily Folvite® Lederle Inc. diluted to suitable strength with physiological saline) and small vitamin B_{12} doses was given along the lines suggested by Hansen and Weinfeld (8)

Case report

The patient was a Finnish girl aged 19 years who for rather more than five months before being seen had been domiciled in Sweden, working as a domestic servant in a sister's household. During the last 6 months she had become increasingly tired and pale but had been working all the time apart from a fortnight five months ago when she had been bedridden with measles followed by sore throat fever and transient arthralgia. She had lost 7 or 8 kg in weight over some six months and attributed this partly to her deliberate attempts to reduce her rather marked corpulence, partly to lack of appetite in recent months. For a few days before admission she had felt feverish and exhausted and had mild pains in the lower abdomen. Her menses were normal. The patient lived in moderately good socio-economic circumstances. Her dietetic history had no remarkable features. Apart from moderately pronounced constipation for many years, she had never had any gastrointestinal disturbances her stools had never been excessively loose or bulky

Physical examination disclosed a fairly plump girl who was febrile and very pale and exhausted. Her skin, oral cavity throat, superficial lymph nodes, lungs and abdomen appeared normal. The spleen and liver could not be palpated. Gynecological examination revealed nothing pathological. The eyegrounds were the site of peripapillary striate hemorrhages and effusions. All tendon reflexes were weak but bilaterally similar the Achilles reflex was absent on both sides. Her vibratory sensibility was normal. The rectal temperature was 39° C.

Laboratory findings Hb 3 7 g% erythrocytes 8×10^5/mm³ hematocrit 10 % mean corpuscular volume 131 μ³ mean corpuscular hemoglobin 48 $\mu\mu$g reticulocytes 25 000/mm³ leukocytes 4 500/mm³ Though the differential leukocyte count was normal, the blood smear exhibited marked anisocytosis, moderate poikilocytosis and 5 to 13 normoblasts/200 leukocytes. Platelets 60 000/mm³

The bone marrow (day 1) was rich in cells. The erythropoiesis exhibited excessive vigour in relation to the immature myeloid cells, a shift to the left and *a uniformly megaloblastic character* An increased incidence of mitoses and karyorrhexis were present. The myelopoiesis was characterized by a nearly normal maturity index. The myeloid cells included 18 % typical giant forms. Specific staining for iron disclosed 72 % sideroblasts, occasional siderocytes and reticular iron of grade ++

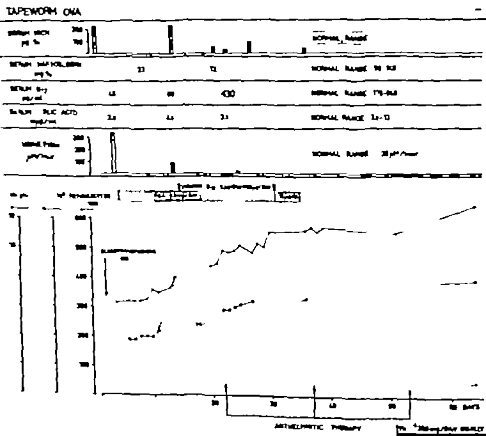


Fig. 1. Excellent effect of "diagnostic" folic acid doses (PGA) on peripheral blood values and urinary FIGlu excretion; minor reticulocyte response to subsequent course of "diagnostic" vitamin B_{12} doses.

Serum electrophoresis yielded normal pattern. E.S.R. 60 mm (Cloudy) Serum bilirubin 1.1—0.7 mg%. Plasma creatinine 1.0 mg%. The urinary sediment contained numerous leukocytes and bacteria on admission but had become normal four days thereafter. The gastric juice contained free HCl in normal amounts. Diphyllobothrium latum (fish tapeworm) eggs and segments were found in the feces, which were negative in Weber' test. X-ray examination of the lungs disclosed no abnormalities.

The *whole blood* folic acid level was 33 mμg/ml, i. e. near the lower limit of normal variation. Data for the folic acid, vitamin B_{12}, haptoglobin and iron concentration in serum as well as for the urinary FIGlu excretion after oral histidine loading have been plotted in fig. 1.

The diagram reveals that the serum folic acid and vitamin B_{12} levels were markedly subnormal, despite the fact that the patient had received two blood transfusions 48 hours before the specimen for making these determinations was drawn.

The patient was put on the regular hospital diet. Two blood transfusions were administered the day after admission. As from her fourth day in hospital the patient was given penicillin in daily dosage of 600,000 I.U. the patient being non-febrile the very next day. Treatment with folic acid 0.2 mg per day subcutaneously was begun on day 4. Two days later the reticulocytes began to increase in number and after twelve days folic acid therapy (i. e. day 15) the reticulocyte count had risen from 25,000 to 530,000 and the

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sorption (day 27) — serum iron estimations made one and two hours after ingestion of 60 mg iron as ferrous sulphate — showed normal values. The folic acid absorption on day 37 lay in the low normal range (maximum serum level 44 mμg/ml). The total fecal fat excretion during 3 days on a high-fat diet was 17 g (in the normal range). The patient was moderately constipated during her entire stay in hospital.

Discussion

In the case of megaloblastic bothriocephalus anemia just described the presence of folic acid deficiency could be demonstrated by three different methods, namely direct serum folic acid estimation, determination of urinary FIGlu excretion after oral histidine loading and administration of "diagnostic" folic acid doses.

The specimens in which the *serum folic acid estimations* were made had been drawn 48 hours after administration of two blood transfusions. However if clinically significant folic acid deficiency is present, the small amount of plasma folic acid supplied with the transfused blood will be metabolized very quickly. Hence the serum folic acid level as a rule correctly reflects the state of the bodily folic acid stores, regardless of whether a transfusion has been given.

Whereas our patient's serum folic acid level was clearly subnormal, her whole blood folic acid level was normal. The most likely explanation here would seem to be that transfused blood corpuscles maintain their folic acid activity practically unchanged as long as they are circulating in the recipient's vascular system (9). Considering that approximately half the corpuscles in the analyzed blood sample would have been donor corpuscles with normal folic acid activity the observed whole blood folic acid level (35 mμg/ml) ought to be regarded as erroneous and too high.

The serum folic acid level is a better criterion than the whole blood folic acid level in the differential diagnosis of megaloblastic anemias (10). For in simple vitamin B_{12} deficiency the serum folic acid level is not seldom abnormally high (over 14 mμg/ml) while the whole blood folic acid level may be subnormal. The mechanism underlying this discrepancy is probably that vitamin B_{12} deficiency deprives the erythroblasts of their ability to incorporate in the normal way some folic acid derivatives, which instead merely accumulate in the serum (8, 9). The increased hemolysis accompanying vitamin B_{12} deficiency states might further contribute to the rising serum folic acid concentration.

Normalcy of the urinary FIGlu excretion is dependent mainly on the maintenance of normal folic acid stores in the liver but the histidine metabolism may also be disturbed, accelerating the FIGlu excretion, in simple vitamin B_{12} deficiency (20). In a series of patients assembled by one of us (H.A.H.) the urinary FIGlu excretion was abnormal in all the 35 with established folic acid deficiency as well as in 16 of 39 with simple vitamin B_{12} deficiency, the excretion rate being markedly accelerated (over 100 μM/h) in 6 of the latter subjects.

The rapid normalization of the urinary FIGlu excretion when the present patient was treated with *small* doses of folic acid suggests that the main cause of the disturbed histidine metabolism was folic acid deficiency. In simple vitamin B_{12} deficiency therapy with *small* folic acid doses has little if any effect on the urinary FIGlu excretion (8).

After 10 to 12 days treatment with small "diagnostic" folic acid doses the

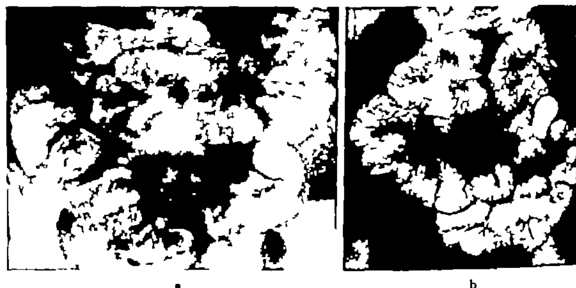


Fig 2. Barium meal examination (a) before and (b) after anthelminthic therapy

hemoglobin concentration and erythrocyte count showed pronounced improvements (cf fig 1) Over the same period the urinary FIGlu excretion per hour dropped from 322 to 97 4 μM/h. The bone marrow obtained by sternal puncture on day 14 exhibited substantial normalization of the morphological picture. Now the erythropoiesis was normo-macroblastic but the myelopoiesis still included 3 giant forms.

The serum vitamin B_{12} level remained low The serum ascorbic acid level was 0 47 mg% (normal range for the time of the year 0.25—0 5 mg%) The transferrin concentration was 390 μg% The xylose excretion was marginally normal (4 8 g in 5 hours)

The patient's general condition showed a dramatic improvement as early as 4 to 5 days after admission, and from then on she was in good spirits. The eye-ground changes were rather less extensive after 11 days folic acid therapy From day 16 a daily supplement of 5 μg vitamin B_{12} was given parenterally After 4 to 5 days vitamin B_{12} therapy the reticulocyte count — which had shown a falling tendency — again began to rise, attaining a new though much less pronounced peak on the tenth day of vitamin B_{12} (and folic acid) administration. The hemoglobin concentration and erythrocyte count also rose but, probably owing to the development of an iron deficiency state (cf fig 1) not to normal levels. No hemorrhage or effusion could be seen in the eye-grounds after 11 days of combined folic acid and vitamin B_{12} treatment.

Barium meal examinations of the gastro-intestinal tract were made on days 15 and 17 the X-ray appearances on the two occasions being similar In the small bowel the jejunum and oral two-thirds of the ileum were rather markedly distended and contained plenty of matter which in parts had a peculiar filamentary appearance. The barium shadow thus exhibited a great number of closely spaced, thread-like voids. Many of these were very narrow only a millimetre or two. Here and there the voids were broader measuring over 3 mm. Occasional convoluted stripes of low density about 3 or 4 cm long and a cm wide were present (cf fig 2 a) The transit time through the small bowel lay within the normal range, the barium meal reaching the oral part of the colon in 3 and 1/2 hours the first and 2 and 3/4 hours the second time.

Anthelminthic treatments were administered on days 22 (9 g extractum filicis) 37 (2 g chloroquine sulphate) and 53 (0.2 g deserpidin) Though the tapeworm never was recovered from the stools, the first treatment seemed to have been successful because subsequently neither eggs nor worm segments could be found in repeated fecal samples and at a third barium meal examination (day 64) the aforementioned characteristic appearances were conspicuous by their absence (fig 2 b). A rough, semi-quantitative test for iron ab-

connected voids in the contrast medium, a picture that agrees only up to a point with that observed in our patient (2, 11 18) However considering that the appearance of the small bowel was normal at barium meal examinations after anthelminthic therapy not only the centimeter wide voids but also the thread-like voids in the contrast medium (fig 2 a) must necessarily have had something to do with the fish tapeworm. A fish tapeworm is rather broad but flat. Hence it should theoretically be outlined most distinctly on an X ray film if it is so located that the rays strike it edge-on, as illustrated in fig 3. Accordingly we feel that the coarser of the thread-like voids probably were caused by the tapeworm lying curled up in the bowel. What caused the very narrow voids seems less clear They could have been due to some filamentary mucous coating formed in response to irritation of the intestinal mucous membrane by the tapeworm.

No tapeworm was observed in the stools following the courses of anthelminthic therapy But the stools contained no *visible* worm in nearly half the cases in a comprehensive series of tapeworm carriers treated with 0.2 g desaspidin although the results of examination for ova a week to a fortnight later were similar to those in cases in which anthelminthic therapy did lead to visible evacuation of the worm (22) Consequently the absence of visible tapeworm evacuation in our case does not disprove either the diagnosis or the effectiveness of therapy

In the light of the case described in the foregoing and of those observations reported in the literature suggesting that folic acid deficiency is present in some carriers of the fish tapeworm, *viz.* retarded erythropoietic and myelopoietic maturation despite a normal serum vitamin B_{12} level (17) a low urinary folic acid excretion (14) and abnormal FIGlu excretion (5) there are good grounds for studying the folic acid metabolism in cases of megaloblastic tapeworm anemia.

Should laboratory facilities for more specific tests for folic acid deficiency not be available, parenteral administration of small "diagnostic" doses of folic acid can be used for demonstrating *ex juvantibus* the presence of any folic acid deficiency

Summary

A case of megaloblastic tapeworm anemia in a girl aged 19 is reported. Her serum folic acid and serum vitamin B_{12} levels were abnormally low and her urinary formiminoglutamic acid (*FIGlu*) excretion after l-histidine loading was considerably increased. Parenteral treatment with "diagnostic" folic acid doses (0.2 mg daily) almost normalized the bone marrow picture within two weeks brought the *reticulocyte* count to optimal values, and reduced towards normal levels the FIGlu excretion, indicating that folic acid deficiency was mainly responsible for the megaloblastic anemia and the abnormal FIGlu excretion. The secondary but much less prominent reticulocyte peak associated with subsequent parenteral administration of "diagnostic" vitamin B_{12} doses (5 μg daily) demonstrates that vitamin B_{12} deficiency was a subordinat etiologic factor Since signs of malabsorption of other complicating disease were absent and several absorption tests after anthelminthic therapy were normal, the fish tapeworm (Diphyllobothrium latum) infestation presumably caused the folic acid deficiency The barium meal examination disclosed the worm located high up in the jejunum and oral part of the ileum. Study of folic acid metabolism



Fig 3 X ray picture of fish tapeworm immersed in barium suspension in plastic tube, showing how worm sections illuminated edge-on are distinctly outlined whilst sections illuminated broadside-on are only faintly perceptible as a broad band (arrow)

present patient's response was an enormous reticulocytosis (fig 1) and almost complete normalization of the morphological bone marrow picture From this we may infer that her megaloblastic anemia was due to deficiency of folic acid (and not of vitamin B_{12}) because we know that, unlike anemias due to vitamin B_{12} deficiency megaloblastic anemias due to folic acid deficiency promptly show optimal hematologic remission in response to *small* doses of folic acid (8 15)

Since small "diagnostic" doses of vitamin B_{12} (3—5 μg daily) possess a corresponding therapeutic specificity (8, 21) administration of small doses of folic acid (0.2 mg daily) and small doses of vitamin B_{12} (3—5 μg daily) may be utilized as a simple and valuable adjunct in the differential diagnosis of megaloblastic anemias (8 15)

Compound folic acid and vitamin B_{12} deficiency states in which the primary factor limiting erythropoiesis is folic acid deficiency may be refractory to vitamin B_{12} therapy despite the presence of vitamin B_{12} deficiency Consequently when such compound deficiencies are suspected treatment should be initiated with small "diagnostic" doses of folic acid

The fact that vitamin B_{12} deficiency constitute a contributory factor in the case reported here is apparent from the second reticulocyte peak and the attendant rise in hemoglobin concentration obtained in response to supplementation of the therapy with small doses of vitamin B_{12}.

Because the blood picture may be normalized by *large* doses of folic acid and/or vitamin B_{12} regardless of whether folic acid and/or vitamin B_{12} deficiency is present, such treatment lacks diagnostic value.

The findings discussed in the foregoing may be summed up by the statement that the patient suffered from a combined deficiency of folic acid and vitamin B_{12}, the folic acid deficiency apparently being the dominant factor in the causation of the disease.

The patient showed neither clinical nor laboratory signs of malabsorption or other complicating disease, so the cause of the folic acid deficiency state was in all probability the roentgenologically massive fish tapeworm infestation *reaching high up into the jejunum*

The extraordinary X ray picture calls for some comment. In the comparatively few papers on the appearance of a tapeworm at a barium examination it is stated that the tapeworm is visualized as a series of comparatively broad inter

connected voids in the contrast medium, a picture that agrees only up to a point with that observed in our patient (2, 11 18) However considering that the appearance of the small bowel was normal at barium meal examinations after anthelminthic therapy not only the centimeter wide voids but also the thread-like voids in the contrast medium (fig 2 a) must necessarily have had something to do with the fish tapeworm. A fish tapeworm is rather broad but flat. Hence it should theoretically be outlined most distinctly on an X-ray film if it is so located that the rays strike t edge-on, as illustrated in fig 3 Accordingly we feel that the coarser of the thread-like voids probably were caused by the tapeworm lying curled up in the bowel. What caused the very narrow voids seems less clear They could have been due to some filamentary mucous coating formed in response to irritation of the ntestinal mucous membrane by the tapeworm.

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Chlorpromazine Jaundice of Long Duration

By

Kai Nørredam

Administration of chlorpromazine is known to cause hepatic damage with jaundice in about 1 per cent of treated cases. Only rarely is this complication of chlorpromazine therapy prolonged in course. Brick et al. (1) found that in only 12 out of 120 cases had the chlorpromazine jaundice lasted for more than 2 months. Recently Read et al. (4) have reported 17 cases from the literature, adding 4 cases of their own, with a duration of more than 3 months. Ten out of these 21 cases had lasted more than 6 months. A further 4 similar cases have been found in the literature by this author. With the 2 cases described below this makes a total of 16 cases lasting more than 6 months (table I).

It is the purpose of this paper to describe the clinical, biochemical and histological findings in 2 patients with unusually prolonged chlorpromazine jaundice. Besides confirming the findings of earlier workers, the description includes a few additional features of the syndrome.

Submitted for publication February 1, 1963.

Case reports

Case 1. In Nov. 1959 a previously healthy 37-year-old woman was treated for paranoid symptoms with chlorpromazine, 225 mg daily. This was stopped when she became jaundiced 18 days later showing increasing values of serum bilirubin, alkaline phosphatase and glutamic-oxaloacetic transaminase (GOT) in the serum, as well as slightly increased cholesterol level. After 45 days of increasing jaundice, laparotomy was performed to exclude the possibility of extrahepatic occlusion. The extrahepatic bile ducts were found to be normal. Histological examination of biopsy specimen (figs. 3 and 4) showed "intense intralobular cholestasis, with many centrilobular bile plugs, and some bile pigment in the parenchymal and Kupffer cells. The portal areas were moderately infiltrated with lymphocytes, but no eosinophils (Dr. Charl. Johansen).

The course of the disease was complicated by recurring abscesses of the skin and infection of the operation wound with staphylococcus aureus. For a long time the patient had frequent pale stools. The only complaints were some tiredness and periodically intense pruritus.

Eight months after the onset of jaundice, she developed increasing xanthomatosis of

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The course of the disease was complicated by recurring abscesses of the skin and infection of the operation wound with staphylococcus aureus. For a long time the patient had frequent pale stools. The only complaints were some tiredness and periodically intense pruritus.

Eight months after the onset of jaundice, she developed increasing xanthomatosis of

Table I Until now published cases of chronic chlorpromazine jaundice lasting more than 6 months

Authors	Sex	Duration of Jaundice in months
Arima & Ogle	?	7
Albacete et al	♀	9
Stacey et al.	♀	7
Gebhart et al.	o	10
	♀	10
Myers et al. (3)	♂	13
Read et al. (4)	♀	7
	♀	7
	♀	18
	♀	36
Beattie et al.	♀	8
Kohn & Myerson	♂	10
Hoffbauer	♀	11
Cares et al	♂	12
The author	♀	19
	♀	15

Pregnant woman.
Significant xanthomatosis of the skin.

the skin in the form of xanthelasmata of the eyelids, yellow colouring of the palms and diffuse miliary xanthomata in the skin. The results of certain biochemical determinations were extremely high and remained so for about 10 months (fig 1) The plasma lipids were raised to more than 10 000 mg/100 ml, most of this being cholesterol and phospholipids. Only 10—20 % of the cholesterol was esterified. Serum bilirubin increased to 15—20 mg/100 ml The alkaline phosphatase was between 150 and 200 KA units/100 ml. The prothrombin was raised to about 150 % o normal. The ESR was continuously between 120 and 140 mm/hour There was no eosinophilia. X ray studies showed numerous small, sharply defined erosions in the bones of the foot and hand (fig 2)

A needle biopsy of the liver 8 months after the onset of jaundice showed decreasing cholestasis of the bile capillaries. In the lobuli, scattered focal necroses could be seen. The portal areas were still slightly infiltrated with lymphocytes (Dr Charl. Johansen) (figs. 5 and 6)

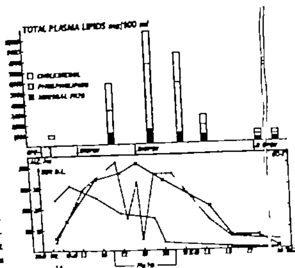
Finally after about 10 months, the serum bilirubin began to decrease slowly The values for serum lipids, alkaline phosphatase and transaminase began to fall 2—3 months later At the last follow-up in May 1962, i.e. 29 months after the onset of jaundice and about 10 months after the jaundice had ceased, the laboratory findings were total bilirubin in serum 0 8 mg/100 ml GOT and GPT normal alkaline phosphatase 11.2 KA units/100 ml total plasma lipids 1,020 mg/100 ml, including 247 mg/100 ml phospholipid and 352 mg/100 ml cholesterol, of which only 19 % was esterified, thymol turbidity normal prothrombin 79 % ESR 36 mm/hour The urine contained no bilirubin or urobilin Clinically the patient looked well and had no symptoms. The only abnormal finding was xanthelasmata around the eyes. There were no clinical signs of cirrhosis.

In July 1961 when the patient had made a complete clinical recovery and when the laboratory findings were only slightly abnormal, a third needle biopsy of the liver was obtained. Histological examination showed "moderately increased fibrosis of the portal tracts with a slight infiltration by lymphocytes and neutrophils. Several fibrous patches were scattered in the parenchyma, apparently corresponding in position to the former focal necroses (Dr Charl. Johansen) (figs. 7 and 8)

The patient was treated with prednisone to no effect. She was also treated with an antihistaminic, with an antiserotonin and with a diet poor first in fats and later in cholesterol. None of these measures had any effect.

In several ways this case is illustrative of iatrogenic diseases. Not only did a prescribed drug induce a protracted damage of the liver she was also submitted to an operation, complicated by an extensive infection of the skin with staphylococcus aureus. Finally the second liver biopsy was followed by a severe intraperitoneal hemorrhage.

Case 2 This patient is a 35-year-old woman. During the past 10 years she had had attacks of cholelithiasis, but had never been jaundiced. Because of paranoid symptoms she was



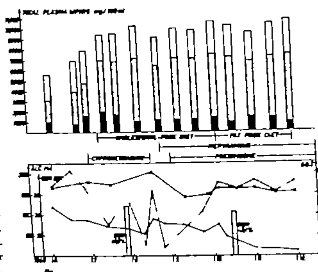


Fig. 1 A. Case 1. The graph show the more important biochemical values up to 2 1/2 years after the onset of illness.

——— = alkaline phosphatase, King Armstrong units/100 ml. —·— = serum bilirubin. — — = GOT = serum glutamic-oxalacetic transaminase units/ml (method of Laursen et al. (7)). Normal range 0.5—1.7 units/ml. CPZ = chlorpromazine administrated for 18 days. The total plasma lipids reach well above 10,000 mg/100 ml. The values of alkaline phosphatase and GOT decrease almost simultaneously but long time after the decrease in serum bilirubin. At the last follow-up (June 1962) all values were normal, except those for the still slightly elevated plasma lipids.

Fig. 1 B. Part of fig. 1 A on larger scale. BSR = bromsulphalein retention test (maximal normal retention 6 %). A third BSR was planned, but abandoned because of allergy to the drug. The total plasma lipids are raised to more than 10,000 mg/100 ml for about 6 months, the maximal value being 10,600 mg/100 ml. Most of this cholesterol and phospholipids. The GOT values show peculiar zig-zag course. Each spike may represent an exacerbation of liver cell necrosis. The course of the disease clearly unaffected by treatment with cyproheptadine (antiserotonin, antihistamine) or mepyramine (antihistamine) or prednison, or by cholesterol-free and fat-free diet.

treated in Nov. 1959 with chlorpromazine, 500 mg daily. Seven days later she had fever and symptoms that were diagnosed as influenza, and the chlorpromazine was discontinued. A few days later she showed dark urine, light stools and then increasing jaundice. The only complaint was intense pruritus. Clinical examination revealed nothing abnormal; there was no swelling of liver or spleen. One month after the onset of jaundice, the icterus index was 100 (normal under 10) and the alkaline phosphatase had risen gradually to 67.5 KA/100 ml. The amylase in the urine was raised to 400 units (normally under 150) at the beginning of the icteric period. The urine generally contained bilirubin, but not always. The excretion of urobilin in the urine also fluctuated; usually there was no excretion of urobilin. Electrophoresis of serum showed slightly increased α_2- and β-fractions. The prothrombin fell to 48 % of normal, but was restored to 100 %

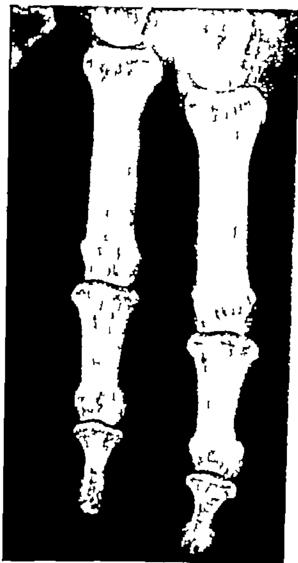


Fig 2. Case 1 The X ray shows many punched-out lesions, presumably xanthomas of the bone. An X-ray control 2 months after serum bilirubin had become normal, and when serum cholesterol was only slightly elevated, showed that the bone lesions had almost, but not quite, disappeared.

after administration of vitamin K. The patient began to have diarrhoea, and the stools were always light-coloured.

As no improvement had been obtained after 3 months of jaundice, a laparotomy was performed. The gall-bladder was found to be filled with stones. However there was no stone in the extrahepatic bile ducts or any dilation of these. Histological examination of liver tissue, obtained during operation, showed "slight infiltration of the portal areas with lymphocytes and plasma cells. There were several small foci of neutrophils in the parenchyma, pronounced cholestasis of the bile capillaries, especially centrilobularly and some bile pigment in the parenchymal cells (Dr V Eskelund) (fig 9)

In June 1960 i. e. 7 months after the onset of jaundice, she had a serum bilirubin of 3.8 mg/100 ml. Alkaline phosphatase was 60.9 K.A units/100 ml, GOT 43, and GPT 100 units/100 ml (method of Karmen). Electrophoresis of serum showed decreased albumin, and slightly elevated values for α_2- and β-globulin. In Aug. 1960 the laboratory findings were essentially unchanged, except that the jaundice had deepened, so that the icterus index was now 60. During this time she had developed xanthelasmata of the eyes.

During the subsequent months the jaundice decreased slowly but it never disappeared completely During this period she complained of dark urine, anorexia, pains in the right hypochondrium and frequent stools. However she was able to stay at home, and to work.

In Feb. 1961 for no apparent reason, she became clearly jaundiced again. However the jaundice disappeared in the course of a few weeks and did not reappear Laboratory findings were icterus index 12—8, alkaline phosphatase 17.5—16.7 BL units/l (normally under 2 3) The stools contained 7 g of fatty substances per day (normally 1—7 g per day). She was treated with a fat-free diet with good effect on her dyspepsia.

At the last follow-up, in July 1962, i.e. 32 months after the onset and 17 months after the cessation of jaundice, she complained only of some tiredness and of slight pains in the right hypochondrium at times. Now the stools were normal. She had xanthelasmata, but no other sign of xanthoma. There was no hepato-splenomegaly She presented no signs of hepatic cirrhosis. The ESR was 27 mm/hour The weight was fully restored, after a loss of weight of 11 kg during the disease. The prothrombin was 82 %, alkaline phosphatase 17.2 BL units/l, the GPT 1.58 (normally less than 0.44) and the GOT 4 0 (normally less than 2.0) The bromsulphalein retention test showed retention of 21.9 mg/l (normally less than 2 mg/l) Total lipids in serum were 1 130 mg/100 ml, including 294 mg/100 ml phospholipid and 321 mg/100 ml cholesterol,



Fig. 3

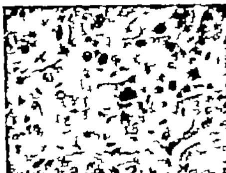


Fig. 4



Fig. 5



Fig. 6



Fig. 7



Fig. 8

Fig. 3. Case 1. The normal structure of the liver cells has been destroyed. Many of the liver cells are degenerating, and fairly extensive focal necroses are seen. The parenchyma is infiltrated with inflammatory cells, constituting small foci in some places. There is a well-marked bile stasis in the centre of the lobules with much bile pigment in the Kupffer and the parenchymatous cells. This specimen was obtained during laparotomy after 45 days of jaundice. Eosin-haematoxylin-stain.

Fig. 4. The same specimen at greater magnification showing severe bile stasis with several bile plugs. Some bile pigment is seen in Kupffer and parenchymatous cells. Many liver cells show degenerative changes, and some unicellular necroses are seen. The sinusoids appear dilated (Eosin-haematoxylin-stain.

Legends to figs. 5–8, see next page

Fig 5. Specimen obtained by needle biopsy 8 months after onset of jaundice The liver cells are profoundly changed over wide area, with clear ballooned cells of which many show nuclear pyknosis. These changes are probably pre-necrotic Some cells have big dark nuclei as sign of regeneration. In one area there is a heavy infiltration with inflammatory cells The bile stasis has now nearly disappeared. The lobular structure remains destroyed (Eosin haematoxylin-stain)

Fig 6. The same specimen at greater magnification. Marked necrosis of liver cells is seen. Only a few bile plugs are now left (Eosin-haematoxylin-stain)

Fig 7 This specimen obtained by needle biopsy 21 months after onset of jaundice and nearly 3 months after the serum bilirubin had become normal, by which time the patient was clinically cured. The normal structure of the liver lobuli has been restored. The liver cells seem normal. The portal areas are invaded by an increased amount of connective tissue with many cells and blood-vessels (van Gieson stain)

Fig 8. The same specimen at greater magnification. The vena centralis is surrounded by connective tissue which sends out fine collagenous fibers between the liver plates (van Gieson stain).



Fig. 9. Case 2. Specimen obtained by operation 3 months after onset of jaundice. Pronounced centrilobular bile stasis is shown, with formation of bile plugs and storage of bile pigment in the Kupffer cells. In the centre of the plate is the vena centralis, surrounded by necrotic layer. T the right, focal necrosis is seen. Small scattered foci of inflammatory cells are seen. Bottom, left, is part of portal area, infiltrated with cells, mostly polymorphonuclear. (Eosin-haematoxylin-stain)



Fig. 10. Case 2. This specimen was obtained by needle biopsy 32 months after onset, and 17 months after the final disappearance of jaundice. A portal area is seen, which is filled with connective tissue, rich in cells and blood-vessels. This tissue seems to spread in an aggressive manner into the adjoining parenchyma, which is seen to the right, top and bottom (van Gieson stain)

of which 79 % was esterified. Electrophoresis showed somewhat reduced albumin, all other fractions being slightly elevated.

At this time needle biopsy was performed. Histological examination showed "no cholestasis in the bile capillaries. Some liver cells contained small amounts of bile pigment. The portal tracts were moderately infiltrated by lymphocytes and variable amounts of eosinophils, showing tendency to attack on the neighbouring lobular parenchyma. In many of the tracts transition into fibrous tissue was seen, in some places extending into the nearby parenchyma. The lobular structure was intact. The fibrosis was moderate and could not be called cirrhosis. In the parenchyma small foci of fibroblasts were seen, evidently replacing former small focal necroses (Dr H. Olesen) (fig 10).

Discussion

From the studies of the authors mentioned, especially that of Read et al. (4) it has become apparent that chronic chlorpromazine jaundice is a well defined clinical, biochemical, and histological entity. This view is supported by the description of the 2 patients in this paper

The syndrome is characterized by its prolonged course, the jaundice lasting up to 36 months and the pathological biochemical values many months more. During the disease the patients feel well, apart from pruritus, which may be distressing and some tiredness. The condition may be complicated by steatorrhoea leading to the malabsorption syndrome. This occurred in both the patients of this study There is almost always some degree of hepatomegaly and sometimes also splenomegaly A pronounced loss of weight is characteristic of the disease.

An interesting feature is the xanthosis that seems to occur when the jaundice lasts for more than 6 months these cases show unusually high values for plasma lipids. The xanthosis may vary from the presence of a few xanthelasmata to an overwhelming diffuse xanthosis, as in Myers case (3)

As a rule the serum bilirubin is elevated up to even more than 30 mg/100 ml. Further it seems that high values of alkaline phosphatase characterise the disease. The most remarkable biochemical disorder is the extremely high value of plasma lipids. In the 4 cases of Read et al the maximal serum cholesterol values varied between 1 120 and 1 825 mg/100 ml. In Myers patient who had an extreme xanthosis, the serum cholesterol was 2,500 mg the serum phospholipids 4,800 mg and the total serum lipids 9 000 mg/100 ml. The patient designated no 1 of my study is unique in this respect in that she shows a serum cholesterol of 3 300 mg serum phospholipids of 6 400 and total plasma lipids of 10 600 mg/100 ml. Both Myers and I were impressed by the fact that the plasma from these patients was completely clear in spite of the enormous amounts of lipids present. This is probably explained by the ability of phospholipids to adhere to the cholesterol molecules and form a colloid solution.

As a rule the amounts of transaminase are moderately elevated Interesting in this respect is the sudden, short rise in GOT seen in patient no 1 (fig 1 B) It might well be that these spikes represent acute exacerbations of liver cell necrosis.

Though it remains unchanged in the milder cases of chlorpromazine jaundice the electrophoretic pattern in the chronic cases invariably shows elevation of the α_2 and β-fractions. Probably this reflects the high concentration of plasma lipids.

Except during a short period in case 2 of this study the thymol turbidity and the flocculation tests were negative. This has been found by other authors also

The raised values of prothrombin concentration in patient no 1 were impressive. In Myers case the prothrombin was 500 per cent the proconvertin and the proaccelerin 300 per cent each Myers has shown that the extreme hyperlipaemia is caused by an increased hepatic production of cholesterol and phospholipid. He suggests that a similar mechanism might be responsible for the elevated values of plasma coagulation factors.

An analogy with primary biliary cirrhosis, where hyperlipaemia is often followed by xanthomatous lesions of the bones, led to an X ray examination of patient no 1 The film showed many punched-out lesions of bone, particularly in the fingers and feet (fig 2) Probably these were xanthomas, although this has not been proved since no biopsy was done.

The histological lesions of the liver in the 2 patients were much alike. Also, they were very similar to those found in

he cases of Read et al. and of Myers. The main findings were three. First there was infiltration of the portal areas by inflammatory tissue, mostly consisting of lymphocytes with a variable amount of eosinophils and with many blood vessels. The portal tracts tended to enlarge into the neighbouring parenchyma, though only to a moderate degree. However I entirely agree with the concept of Read et al. that the histological lesions in this respect are closely related to those seen in primary biliary cirrhosis. Secondly the cholestasis of the bile capillaries. This was not different qualitatively from that seen in mild cases of the disease. In the chronic cases of this study the intrahepatic cholestasis seemed to decrease rather early and at a time when the other lesions were still pronounced. Thirdly lesions of the parenchyma in my cases many unicellular and focal necroses were seen. Furthermore, in both patients I found necrosis of several layers of liver cells around the central veins. This has not been described before. In Myers case the liver cells were light and ballooned. This was seen in the present study also, particularly in the second biopsy of patient no 1 (figs. 5 and 6) Myers believed these to be xanthomatous cells. Unfortunately in none of the cases found in the literature, including those of this paper has special staining for cholesterol or phospholipids been applied The ballooning might be sign of incipient necrosis, since many of these cells presented distorted nuclei or had lost their nuclei completely

The results reported here are in full agreement with the statement of Read et al. that chronic chlorpromazine jaundice always show remarkable regression, linically as well as biochemically and histologically

This does not mean however that completely normal conditions are restored. In case no 1 the last biopsy taken about 6 months after the jaundice had ceased showed moderate fibrosis, particularly of the portal areas. In case no. 2 the last biopsy taken 16 months after the jaundice had disappeared also showed fibrosis of moderate degree (figs. 7—8 and 10) The histological findings were in this respect similar in Myers case, in which biopsy was taken 9 months after the jaundice had ceased.

At the time when biopsy showed this fibrosis, i. e. many months after serum bilirubin had become normal patient no. 1 presented normal values of GOT and alkaline phosphatase, but slightly elevated values of serum lipids and ESR. In the same phase of her disease patient no 2 had slightly raised values of GOT GPT alkaline phosphatase bromsulphalein retention serum lipids and ESR.

It can be stated that chronic chlorpromazine jaundice is generally perhaps always, followed by fibrosis of the liver of a slight to moderate degree. This should not be called cirrhosis, since the lobular structure of the liver remains intact. The important and interesting question arises what will happen to this fibrosis? Will it remain unchanged, will it disappear or will it proceed to a clear-cut cirrhosis in the manner of primary biliary cirrhosis? I have seen only one suggested answer to this question, viz. by Zelman (5) in whose case biopsy of the liver after chronic chlorpromazine jaundice of 4 months duration showed some degree of fibrosis. 3 years later a second biopsy showed no fibrosis.

However it seems probable that a final answer to this question must wait for some years.

The syndrome is characterized by its prolonged course the jaundice lasting up to 36 months and the pathological biochemical values many months more. During the disease the patients feel well apart from pruritus which may be distressing and some tiredness. The condition may be complicated by steatorrhoea leading to the malabsorption syndrome. This occurred in both the patients of this study There is almost always some degree of hepatomegaly and sometimes also splenomegaly A pronounced loss of weight is characteristic of the disease.

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From Medical Department, Nordland County Hospital, Bodö, Norway

Clinical Considerations in Quinidine Therapy of Chronic Auricular Fibrillation

A Study of 200 Unselected Patients — with Follow-up

By

Rolf Rokseth

There is no unanimous agreement on the indications and the results of quinidine treatment in chronic auricular fibrillation. Some authors recommend that quinidine should be given to all patients with persistent auricular fibrillation (3, 14, 23) while others have treated selected patients (8, 21). The influence of various parameters, such as etiology of the heart disease, duration of fibrillation etc. is also disputed, partly because of different criteria for selection or because the number of treated patients has been too small.

In a previous paper on syncope (19) we have briefly reviewed the immediate results of quinidine therapy in 274 unselected patients. The object of this paper is to analyze the results in the last 200 patients, especially with regard to factors influencing successful therapy and to the problem of selection. Follow-up examinations were carried out at regular intervals.

Submitted for publication February 1, 1963.

Methods

In the course of 4 1/2 years, 1954 to 1959 all our patients with chronic auricular fibrillation, except those with total A—V block, were treated with quinidine. All had had fibrillation for at least 7 days. Information about parameters to be studied was written down beforehand: age, duration of fibrillation, etiology of the heart disease, degree of congestive heart failure etc. A heart radiogram was taken in most cases.

All patients were observed for some time in our hospital before quinidine was given. All were digitalized and given maintenance doses, usually digitoxin, 0.1 mg daily. The symptoms and signs of congestive heart failure were reduced as far as possible with salt restriction and diuretics. Anticoagulants (dicoumarol) were given to all patients, and quinidine was not started until the PP-level was stabilized between 10 and 30 %.

During quinidine therapy the patients were kept in bed. Dicoumarol treatment and bed-rest were continued for a week after restoration of sinus rhythm. The doses of quinidine sul-

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Summary

Two cases of chlorpromazine jaundice are described which lasted 19 and 15 months respectively. The first patient developed diffuse xanthosis and xanthomas of the bones. Both patients presented highly elevated values of bilirubin, alkaline phosphatase and transaminase in serum, and the first patient had an extreme hyperlipaemia.

Repeated histological examinations of liver tissue showed intrahepatic cholestasis, scattered necroses and inflammative infiltration of the portal areas. The latter tended to infiltrate the surrounding parenchyma, imitating the early stage of primary biliary cirrhosis.

Clinically the disease showed complete spontaneous remission. However many months later both patients still had biochemical signs of hepatic dysfunction, and histologically showed slight to moderate fibrosis of the liver. The ultimate prognosis of the disease, especially as to the hepatic fibrosis, is not known at present.

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Table I Results of quinidine therapy in chronic auricular fibrillation

	No.	Restored	Sinus rhythm On discharge	Sinus rhythm After 6 months
Diagnosis				
Rheumatic heart disease	93	48	37 (39.8%)	28
Arteriosel. heart disease	47	28	22 (46.8%)	16
Hypertensive heart disease	25	11	8	7
Thyrotoxic heart disease	14	8	8	8
Other heart disease	21	12	9	6
Age				
< 50	24	12	9	7
50–59	41	23	21 (51.2%)	19
60–69	79	42	29 (36.7%)	25
> 70	56	30	25 (44.6%)	14
Duration of fibrillation				
< 6 months	47	36	33 (70.2 %)	25
6–24 months	36	20	16 (44.4 %)	14
> 24 months	107	48	32 (29.9%)	23
Unknown	10	3	3	3
Functional group				
I	5	3	2	1
II	68	49	37 (54.4%)	32
III	69	37	34 (49.3%)	23
IV	58	18	11 (18.9%)	9
Total	200	107	84 (42.0%)	65

phate were 0.3 g × 3, 0.3 × 4, 0.3 × 6, 0.3 × 8, 0.3 × 10, 0.3 × 12, 0.3 × 12 taken orally on successive days. Auscultation of the heart was done at least twice a day and the nurse was instructed to record pulse before each new dose of quinidine. An electrocardiogram was taken at least once a day and when higher doses of quinidine were given, several times a day. Quinidine was discontinued or temporarily reduced in the case of toxic reactions or widening of the QRS complex by more than 25. A maintenance dose of quinidine was always given when sinus rhythm was restored usually half the dose required for conversion, or at least 0.3 × 3 or 4. The patients were re-examined every third month during the first year and later once a year.

Results

The results are seen in table I. Sinus rhythm was restored in 107 patients (53.5 %) and maintained during hospitalization in 84 (42 %).

As regards *etiology* of the heart disease, the success rate was greatest in the small group with thyrotoxic heart disease. There was apparently no notable difference between the other groups. But when patients with congestive heart failure and those with fibrillation of more than 6 months duration were excluded conversion was most successful in the non-

neumatic group There were then 3 immediate failures and 8 successful attempts in patients with rheumatic heart disease as compared to 3 and 23 respectively in the non-rheumatic group. Table II shows that the immediate conversion rate was higher in patients with mitral stenosis than in those with mitral regurgitation.

The difference in conversion rate between the various *age* groups was small, and might have been due to chance.

On the other hand there was a significant decrease in conversion rate with increasing *duration of fibrillation* ($p < 0.001$) This is confirmed by table III where cases with severe congestive heart failure re excluded. The conversion rate decreased distinctly with increasing degree *of congestive heart failure* also when patients with long duration of fibrillation were excluded. Among patients with fibrillation of less than 2 years duration, 42 of 63 in groups I—III and only 7 of 20 in group IV had sinus rhythm on discharge from hospital. The patients were grouped according to the New York Heart Association nomenclature.

T ble IV shows the influence f *heart size* as determined by X-ray Only 155 patients are included partly because a cardiac radiogram was not always taken t the beginning of the study and partly because the measurements n some cases were less accurate because of severe lung congestion pleura exudate, thorax deformity etc. There ppeared to be a decreasing conversion rate with increasing heart size. It was possible, however to restore and maintain sinus rhythm in some patients with fairly large hearts.

The *doses* of quinine and the *duration* of treatment are seen in tables V and VI Si us rhythm was as a rule restored during the first few days and with small doses. More than one week s treatment was needed n 17 patients. Twenty patients were treated two or more times over a period of months to 4 years. Sinus rhythm was restored in 14 of these more than once. Two patients were successfully treated after unsuccessful attempts 6 months earlier

Table II Quinidine therapy in patients with rheumatic heart disease

	Conversion	Failure
Mitral Stenosis	25	15
Mitral Incompetence	7	14
Mitral and aortic valve disease	6	11
Aortic valve disease	10	5

Table III Duration of auricular fibrillation in patients without severe congestive heart failure

	Total	Sinus rhythm On discharge	Sinus rhythm After 6 months
< 6 months	37	28 (75.6%)	21
6—24 months	26	14 (54.0%)	12
>24 months	69	28 (40.4%)	20
Unknown	10	5	5

Table IV Influence of heart size (X-ray) on restoration of sinus rhythm

Heart volume (ml/m² B. S.)	Sinus rhythm Restored	Sinus rhythm Not restored	Sinus rhythm After 6 months
2—400	9	2	4
4—600	41	20	28
6—800	20	19	11
>800	7	15	2
Total	77	56	45
Mean volume	575	700	535

Table V Duration of treatment until restoration of sinus rhythm

	Days	No.
1st week	1	8
	2	21
	3	24
	4	19
	5	10
	6	6
	7	2
2nd week		14
3rd week		2
4th week		1
	Total	107

Table VI Average daily dose of quinidine when sinus rhythm was restored

Grams	No.
0.9	8
1.2	26
1.8	28
2.4	22
3 0	13
3.6	10

Table VII Toxic effects on the heart during quinidine therapy

	No.	Sinus rhythm after 6 months
Bundle branch block	9	0
Ventricula premature beats	6	1
A V block 1st degree	6	4
A V block 2nd degree	1	1
A V block 3rd degree	2	1
Nodal rhythm, tachycardia	11	6
Bradycardia	5	3
Auricular flutter	22	9
Ventricular tachycardia, fibrillation	1	0
Total	63	25

Two patients who had nausea when taking quinidine were given quinine chloride in increasing doses without side effects Sinus rhythm was restored in one of them.

The *complications* were as follows cardiac in 63 patients (table VII) gastrointestinal disturbances in 60 and dizziness, tinnitus, etc. in 20 One patient had a thrombocytopenic reaction, and a possible pulmonary embolus occurred in another

Sudden loss of consciousness occurred in 11 patients, which has been described earlier (19) The causes seemed to be transient respiratory arrest, fall in blood pressure bradycardia or ventricular arrhythmias. All were in group III or IV of congestive heart failure, and many had had fibrillation for more than 2 years. Age, dose of quinidine or duration of treatment did not seem to be important. All patients recovered either spontaneously or after immediate measures such as artificial respiration isuprel sublingually in bradycardia or ventricular arrhythmias or ephedrine hydrochloride intramuscularly when blood pressure fell.

Follow-up studies

Table I shows that 65 (32.5 %) patients maintained sinus rhythm for at least 6 months. Six had relapsed to auricular fibrillation and 13 did not show up for control after 6 months. It is noted that relapse to auricular fibrillation usually occurred early after conversion to sinus rhythm Among 29 relapses, 23 occurred before the patients left hospital. Table VIII shows that etiology of heart disease did not significantly affect maintenance of sinus rhythm. There was a 30 % rate of relapses among patients above 60 years, as against 20 % among patients below 60 Duration of fibrillation

for more than 2 years gave relapses in 41.7 % as against 16.1 % among the patients with a duration of fibrillation less than 2 years. Patients who on admittance were in group IV heart failure also showed a greater tendency to relapse with auricular fibrillation.

It is sometimes stated that when sinus rhythm is difficult to restore it is also difficult to maintain. In our study however 11 of the 17 patients who needed more than a week's treatment with quinidine maintained sinus rhythm for at least 6 months.

There is often doubt as to the advisability of giving quinidine therapy when auricular fibrillation is combined with *bundle branch block*. In 13 of our patients, the QRS measurement was 0.12 secs or more on admittance. Sinus rhythm was restored in 7 but maintained for 6 months in 3 patients only. No serious toxic reactions occurred.

Among the relapses during the observation period after hospitalization, 1 patient had inadvertently been given 0.10 g × 3 instead of 0.5 g × 3 and 2 others had of their own accord discontinued taking quinidine tablets with immediate recurrence of auricular fibrillation. A fourth patient had stopped taking tablets due to a rash from other causes. These experiences point to the necessity of taking a maintenance dose regularly.

Table VIII. Relapses to auricular fibrillation after successful quinidine therapy

	Sinus rhythm restored	Relapse	N control
Diagnosis			
Rheumatic heart disease	48	14	6
Non-rheumatic heart disease	59	15	7
Age			
< 60 years	35	7	2
> 60 years	72	22	11
Duration of fibrillation			
< 2 years	56	9	8
> 2 years	48	20	5
Congestive heart failure			
Group I—III	89	20	13
Group IV	18	9	0

Clinical observations

Improvement after restoration of sinus rhythm was regularly noted and was most pronounced in patients with severe congestive heart failure. This is illustrated by a woman aged 65 with rheumatic heart disease in group IV. Sinus rhythm was restored, she took maintenance doses and managed fairly well at home. After some months she discontinued quinidine on her own, immediately relapsed to auricular fibrillation and developed increasing congestive heart failure. Her diet and digitalis dose were not changed. She was again hospitalized, sinus rhythm was restored a second time, and her cardiac function again improved considerably.

Comment

Hemodynamic examinations have repeatedly shown the advantages of quinidine therapy when sinus rhythm is restored (2, 10, 12, 15, 22). Most outstanding is an increase in cardiac output, especially during exercise. Patients with auricular fibrillation often have a pronounced tachycardia during exercise, which is only partially overcome by digitalis (5, 24). Embolism may occur after conversion but is probably more

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Two patients who had nausea when taking quinidine were given quinine chloride in increasing doses without side effects. Sinus rhythm was restored in one of them.

The *complications* were as follows cardiac in 63 patients (table VII) gastro-intestinal disturbances in 60 and dizziness, tinnitus, etc. in 20. One patient had a thrombocytopenic reaction, and a possible pulmonary embolus occurred in another

Sudden loss of consciousness occurred in 11 patients, which has been described earlier (19) The causes seemed to be transient respiratory arrest, fall in blood pressure bradycardia or ventricular arrhythmias. All were in group III or IV of congestive heart failure, and many had had fibrillation for more than 2 years. Age dose of quinidine or duration of treatment did not seem to be important. All patients recovered either spontaneously or after immediate measures such as artificial respiration, isuprel sublingually in bradycardia or ventricular arrhythmias or ephedrine hydrochloride intramuscularly when blood pressure fell.

Follow-up studies

Table I shows that 65 (32.5 %) patients maintained sinus rhythm for at least 6 months Six had relapsed to auricular fibrillation and 13 did not show up for control after 6 months. It is noted that relapse to auricular fibrillation usually occurred early after conversion to sinus rhythm Among 29 relapses, 23 occurred before the patients left hospital. Table VIII shows that etiology of heart disease did not significantly affect maintenance of sinus rhythm. There was a 30 % rate of relapses among patients above 60 years, as against 20 % among patients below 60 Duration of fibrillation

for more than 2 years gave relapses in 41.7 % as against 16.1 % among the patients with a duration of fibrillation less than 2 years. Patients who on admittance were in group IV heart failure also showed a greater tendency to relapse with auricular fibrillation.

It is sometimes stated that when sinus rhythm is *difficult* to restore, it is also difficult to maintain. In our study however 11 of the 17 patients who needed more than a week's treatment with quinidine maintained sinus rhythm for at least 6 months.

There is often doubt as to the advisability of giving quinidine therapy when auricular fibrillation is combined with *bundle branch block*. In 13 of our patients, the QRS measurement was 0.12 secs or more on admittance. Sinus rhythm was restored in 7 but maintained for 6 months in 3 patients only. No serious toxic reactions occurred.

Among the relapses during the observation period after hospitalization 1 patient had inadvertently been given 0.10 g × 3 instead of 0.3 g × 3 and 2 others had of their own accord discontinued taking quinidine tablets with immediate recurrence of auricular fibrillation. A fourth patient had stopped taking tablets due to a rash from other causes. These experiences point to the necessity of taking a maintenance dose regularly.

Clinical observations

Improvement after restoration of sinus rhythm was regularly noted and was most pronounced in patients with severe congestive heart failure. This is illustrated by a woman aged 65 with rheumatic heart disease in group IV. Sinus rhythm was restored, she took maintenance doses and managed fairly well at home. After some months she discontinued quinidine on her own, immediately relapsed to auricular fibrillation and developed increasing congestive heart failure. Her diet and digitalis dose were not changed. She was again hospitalized, sinus rhythm was restored a second time, and her cardiac function again improved considerably.

Table VIII. Relapses to auricular fibrillation after successful quinidine therapy

	Sinus rhythm restored	Relapse	No control
Diagnosis			
Rheumatic heart disease	48	14	6
Non-rheumatic heart disease	59	15	7
Age			
< 60 years	35	7	2
> 60 years	72	22	11
Duration of fibrillation			
< 2 years	56	9	8
> 2 years	48	20	5
Congestive heart failure			
Group I—III	89	20	13
Group IV	18	9	0

Comment

Hemodynamic examinations have repeatedly shown the advantages of quinidine therapy when sinus rhythm is restored (2, 10, 12, 15, 22). Most outstanding is an increase in cardiac output, especially during exercise. Patients with auricular fibrillation often have a pronounced tachycardia during exercise, which is only partially overcome by digitalis (5, 24). Embolism may occur after conversion, but is probably more

frequent if auricular fibrillation is allowed to continue (3 6 9 17 20) Hecht and Lange (11) state that the cardiac output in some patients remains unchanged after restoration of sinus rhythm It is, however difficult to predict the magnitude of the clinical and hemodynamic improvement in the individual patient It can be assumed that most patients will benefit more or less by obtaining a regular sinus rhythm. The practical question is What are the chances of restoring sinus rhythm in a patient with a particular complex of symptoms and signs, and what are the risks?

The general success rate in our study was not outstanding when compared with many other reports. In materials collected by Holzman and Brown (13) the conversion rate averaged 67 % varying from 31 to 88.5 / Goldman (7) refers to various series with a success rate of 53 to 89 % Many of the reports, however concern selected patients while our material was unselected But also in some unselected series a conversion rate of 75 to 80 % is given (14 26) Besides differences in selection the methods of treatment have also varied Many authors have increased the doses of quinidine more rapidly and/or reached higher doses. Sokolow and Ball (21) however found no notable difference between "rapid and slow administration, but mention that conversion was obtained in 5 patients with the rapid method after the "slow method had failed When large doses were unsuccessful or caused toxic reactions, Kissane et al. (14) stopped the drug for a day or two and then resumed the previous maximum dose after which conversion was often accomplished.

Our success in maintaining sinus rhythm however compares well with figures collected by Holzman and Brown (25 to 38 %) Delayed-absorption coated tablets of quinidine may possibly give a higher maintenance rate (16)

The favourable results in thyrotoxic heart disease confirm earlier reports (7). As regards the other etiological groups there has been considerable disagreement. Some authors have found restoration more difficult in patients with rheumatic heart disease than in those with non-rheumatic heart disease (13 21) Others had more success in patients with rheumatic heart disease (18) A third group (4 26) found the etiology of the heart disease to be without significant influence When the effect of other factors, such as congestive heart failure and duration of fibrillation is excluded our results seem to confirm the unfavourable influence of rheumatic etiology

Yount et al. (26) found that duration of fibrillation and degree of congestive heart failure were of little importance in successful restoration of sinus rhythm. Wolff and White (25) stated that heart size did not influence the success of quinidine therapy It is, however apparent that by our methods restoration and maintenance of sinus rhythm was less successful when duration of fibrillation had been several years, with severe congestive heart failure and with large heart size. A combination of severe congestive heart failure and auricular fibrillation for more than 2 years was found in 36 of our patients. Sinus rhythm was restored in 11 but maintained for 6 months in 3 only Besides syncope occurred in 5 patients, and total A—V block in one other On the other hand Askey (1) collecting large materials, found the natural risk of sudden death in patients with auricular fibrillation and severe congestive heart failure to be only very

slightly less than the risk during quinidi e therapy These patients are in greatest need of treatment, and Askey concludes that quinidine at times may be justified since restoration of sinus rhythm may be life-saving in some patients with long-standing uricular fibrillation and severe congestive heart failure. According to our study careful supervision and immediate, appropriate treatment of respiratory depression, arrhythmias and fall in blood pressure minimize the risk of sudden death ascribable to quinidine.

The value of anticoagulant treatment prior to and during attempts to restore sinus rhythm has not been firmly established by controlled series, and it may be difficult to compare materials from different authors. According to our study however the risk of embolism seems small when anticoagulants are routinely given. Many authors who either gave anticoagulants to selected patients only or who do not state whether anticoagulants were given or not report a higher incidence of embolizations (4 6 8)

Summary and conclusions

The results of quinidine therapy in 200 unselected patients with persistent auricular fibrillation are analyzed. All were digitalized and given anticoagulants before and during quinidine treatment.

Sinus rhythm was restored in 107 patients t was maintained during hospitalization in 84 and for at least 6 months in 65 The conversion rate was greatest in patients with thyrotoxic heart disease and in those with uncomplicated mitral stenosis, but poor in those with mitral insufficiency Significant heart enlargement, severe congestive heart failur and fibrillation of several years duration especially when combined, ad ersibly in fluenced the success, and increased the risk of complications.

The main danger during qu nid ne treatment wa sudden loss of consciousness which occurred in 11 patients. All of them recovered. There were no deaths and only one possible incident of embolism

Bundle branch block was present in 13 patients prior to quinidine therapy and no serious toxic react ons occurred. Seventeen patients required more than one week s treatment for conversion and sinus rhythm was maintained for at least 6 months in 11 of them. Several patients were restored to sinus rhythm more than once in the course of some months to 4 years, and a previous unsuccessful attempt was sometimes followed by a successful one.

E idently it may not be feasible to give rigid criteria for selection of patients for quinidine treatment. When the duration of fibrillation has been short, and congestive heart failure is not severe the indication is usually obvious. In patients with a combination of heart enlargement, severe congesti e heart failure and fibrillation of several years duration the indication for quinidine may be doubted. According to our tudy however apparently serious complications may be successf lly treated provided that adequate precautions are taken, and these patients should not be excluded from quinidine therapy Successful restoration and maintenance of sinus rhythm was possible in some of them and the clinical improvement was then pronounced.

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Anticoagulants in Acute Myocardial Infarction

Recurrent Myocardial Infarction and Death after Discontinuance of Anticoagulant Therapy

By

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The value of long-term anticoagulant therapy in coronary heart disease is still a subject of discussion. Controlled clinical trials seem to have established a beneficial effect in patients with myocardial infarction at least in the first postinfarction period in younger age groups (1 3 4 6) There is, however diversity of opinion about the magnitude of this effect and how long t lasts. In addition a new problem has arisen since it has been claimed that discontinuance of anticoagulant therapy involves an increased danger with accumulation of thrombo-embolic episodes and deaths in the months following probably due to postulated rebound hypercoagulable state (5 7 8)

This paper deals with the effect of discontinuance of anticoagulant therapy one year after myocardial infarction. Our main problems have been, firstly to evaluate whether one year of anticoagulant therapy is sufficient to give the same beneficial effect as permanent treatment, secondly to find out whether discontinuance is followed by an increased number of deaths or recurrent infarctions when the anticoagulant therapy is tapered off over a period of weeks.

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Mat rial and methods

I the Medical Department, Drammen Hospital, all patients with myocardial infarction have been treated with anticoagulants in the acute stage and for about one year afterwards. After this time the anticoagulant therapy has been gradually discontinued over period of 2 weeks except in patients with cardiac arrhythmias, with previous embolism or severe angina pectoris.

During the 3-year period Jan. 1st 1956 to Dec. 31st 1958 total of 414 patients with acute myocardial infarction were treated. Of these 90 died within 4 weeks after the acut infarction and are not included in this investigation. A total of 109 patients were excluded for other reasons (table 1) The rest, 215 patients, received anticoagulant therapy The criteria used for the diagnosis and selection of the patients were the same as those employed by Bjerkelund (1) As guide for the dosage of anticoagulants PP-values (9)

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and post-mortem examination therefore was not carried out.

The survival curves show no obvious change at the time of discontinuance of the anticoagulant therapy (fig 1) With due allowance for difficulties arising from comparing two different materials, we have compared the survival rate in this material with the results of Bjerkelund (1) In patients under 60 years of age the survival curve has nearly the same slope as that of Bjerkelund's patients who had permanent anticoagulant treatment. This may indicate that the method used in our study gives the same protection as permanent anticoagulant therapy in this age group. In patients 60 years or more, however our curve is nearly parallel with that of Bjerkelund's untreated patients, and this is true also in the first post-infarction year when in fact all our patients received anticoagulant therapy These results may perhaps, reflect the unsatisfactory effect of anticoagulant therapy in elderly patients.

Eighteen patients died after discontinuance of the anticoagulant therapy One patient died within four weeks, 3 patients within eight weeks and a total of 6 patients within four months thereafter However this may not be interpreted as an accumulation of deaths in the first months after therapy was discontinued. I fact, in patients in whom the anticoagulant therapy was not discontinued the time of deaths are scattered in exactly the same manner around an arbitrarily chosen point (i.e. 12 months after the acute infarction) There is, therefore, no significant relationship between the time of death and the time of discontinuance of anticoagulant therapy

These results are in accordance with recent report of Bjerkelund (2) who found no increased danger of recurrence

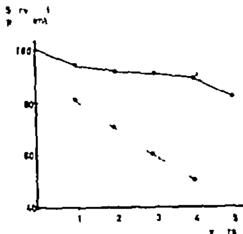


Fig. 1 Survival curves in patients with myocardial infarction aged 60 years or more (stippled curv) and under 60 years Anticoagulant therapy discontinued after one year in 61 out of 115 patients in the older group and in 66 out f 100 patients in the younger group.

of myocardial infarction or in mortality after cessation of anticoagulant therapy when this was tapered off over a period of 4—5 weeks in patients who had been treated for several years. Sise et al. (10) observed rebound" thromboembolic complications significantly more frequently in those patients who stopped the treatment for bleeding than n those who stopped treatment for other reasons. This may account for the accumulation of thromboembolic complications in cases where the anticoagulant therapy has been stopped abruptly fo different or unspecified reasons

It is our opinion that anticoagulant prophylaxis beyond one year after acute myocardial infarction is not justifiabl in the majority of cases. An even shorter period of therapy than one year may eventually prove to be ufficient. A controlled study to evaluate this possibility is in progress.

Table I Selection of patients for the investigation

Total no. of patients with myocardial infarction		414
Death in the acute stage (22%)	90	
Patients over 75 years old	32	
Patients with previous infarction, admitted for other reasons	31	
Diagnosed post mortem	3	
Patients treated only in the acute stage	43	199
Total no. of patients for investigation		215

Table II Age and sex distribution. The average age of all patients is 62 years. The numbers in brackets refer to patients in whom anticoagulant therapy is discontinued

Age (yrs)	Men	Women	Both sexes
<60	90 (65)	10 (3)	100 (66)
60 or >	93 (49)	22 (12)	115 (61)
Total no. of cases	183 (112)	32 (15)	215 (127)

Table III Previous diseases

	No. of patients		
	< 60 yrs	60 yrs or >	Total
Myocardial infarction	10	10	20
Angina pectoris	24	41	65
Diabetes mellitus	6	7	13

were estimated at regular intervals, a therapeutic level being defined as falling between 15 and 30. Efforts were made to give the patients cardiological supervision and treatment apart from the anticoagulant therapy. The patients were observed from 24 to 60 months, or until death. The observation time ended on Dec. 31st 1960.

The distribution of age and sex in the material is shown in table II. Anticoagulant therapy was discontinued in 127 out of 215 patients (59). The average duration of the anticoagulant therapy in these 127 patients was 15 months.

Table IV Number of deaths during the observation period

Causes of death	Total no.	Under anticoag. therapy	After discont. therapy
Sudden death	14	11	3
Recurrent infarction	15	9	6
Heart failure	10	9	1
Ruptured heart	1	1	0
Cerebral apoplexy	4	1	3
Subarachnoid haemorrhage	1	1	0
Pulmonary embolism	2	2	0
Intercurrent diseases	2	1	1
Unknown causes	11	7	4
Total	60	42	18

Results and discussion

Recurrent myocardial infarction

Non fatal recurrent myocardial infarction was diagnosed in 33 patients during the observation period. Of these patients only 10 had discontinued anticoagulant therapy at the time of recurrence. The intervals between the discontinuance and the onset of the new infarction were ½-4-4-4-6-10-19-22-28 and 52 months respectively.

Deaths

A total of 60 patients died during the observation period. 42 patients died during anticoagulant therapy and 18 patients after its discontinuance. The PP values at the time of death in the first group were within therapeutic levels in almost all cases. The causes of death are listed in table IV. The number of deaths classified as being due to "unknown causes" or "sudden death" is comparatively high owing to the fact that many of the patients died outside the hospital

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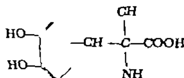
From Menighetssøsterhjemmets klinikk (Head: H. Storm Mathisen, M. D.) Oslo Norway

Clinical Studies with Methyl-dopa (Aldomet®) in Patients with Hypertension During Two Years

By

HAAKON STORM MATHISEN

Increasing attention has focused on the role of cathecholamines in the regulation of blood pressure. Inhibition of decarboxylation and hence of production of noradrenalin and other mono-amines may be achieved by administration of alpha-methyl-dopa (1-alpha-methyl-3 4-dihydroxy-di-phenylalanine) which was synthesized by Stein and Pfister in 1951 (17) The structural formula is



Bourkes (16) found that the substance tested in vitro inhibited decarboxylation of dopa. Dengler and Reichel (1) in 1958 found that premedication with methyl-dopa blocked the pressor effect of dopa in cats and other laboratory animals. Sjoerdsma (15) has studied decarboxylation of several amino acids in hypertensive subjects. H gave the patient amino acid per os or intravenously and measured the urinary excretion of its corresponding amine during the next 8 hours. The experiment was then repeated, but now 2 g of methyl-dopa were given per os 2 hours before the amino acid was administered. This reduced the urinary excretion of amine by 50—80 Gillespie and Sjoerdsma (2) in short-term experiments found that methyl-dopa influenced blood pressure causing it to fall within 48—72 hours after administration of 1 1/2 g When medication was stopped there was a rise in blood pressure within 24 hours. In short-term experiments, Sannerstedt et al. (13) recently have given methyl-dopa to hypertensive patients engaged in measured muscular effort with bicycle-ergometer The result was reduced blood pressure both during rest and at work, slower heart rate but unaltered cardiac output. This indicates that the substance acts by reducing peripheral resistance A reduction in renal vascular resistance under methyl-dopa medication was also found. Recently the effectiveness of methyl-dopa as a hypotensive agent has been demonstrated by Oates et al. (8) and others (2, 3, 4 6 7 10 14 18)

Submitted for publication February 1 1963.

Summary

Two hundred and fifteen patients who had survived the acute stage of myocardial infarction have been treated with oral anticoagulants for at least one year afterwards. After this time the anticoagulant therapy has been gradually discontinued over a period of two weeks except in patients with cardiac arrhythmias previous embolism or severe angina pectoris.

We have found no accumulation of recurrent infarction or cardiovascular deaths in the first period after the discontinuance of anticoagulant therapy Furthermore we have found no change in the slope of the survival curves at the time of discontinuance.

It seems reasonable to conclude that anticoagulant prophylaxis beyond one year after acute myocardial infarction is not justifiable in the majority of cases and that discontinuance of anticoagulant therapy at this time involves no increased risk of thromboembolic complications when the anticoagulant therapy is tapered off over a period of 2 weeks

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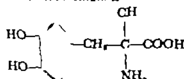
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Clinical Studies with Methyl-dopa (Aldomet®) in Patients with Hypertension During Two Years

By

Haakon Storm Mathisen

Increasing attention has focused on the role of cathecholamines in the regulation of blood pressure. Inhibition of decarboxylation and hence of production of noradrenalin and other mono-amines may be achieved by administration of alpha-methyl-dopa (1-alpha methyl-3,4-dihydroxy-dl-phenylalanine) which was synthesized by Stein and Pfister in 1951 (17). The structural formula is



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Summary

Two hundred and fifteen patients who had survived the acute stage of myocardial infarction have been treated with oral anticoagulants for at least one year afterwards After this time the anticoagulant therapy has been gradually discontinued over a period of two weeks except in patients with cardiac arrhythmias previous embolism or severe angina pectoris.

We have found no accumulation of recurrent infarction or cardiovascular deaths in the first period after the discontinuance of anticoagulant therapy Furthermore we have found no change in the slope of the survival curves at the time of discontinuance

It seems reasonable to conclude that anticoagulant prophylaxis beyond one year after acute myocardial infarction is not justifiable in the majority of cases and that discontinuance of anticoagulant therapy at this time involves no increased risk of thromboembolic complications when the anticoagulant therapy is tapered off over a period of 2 weeks.

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pressure was found reduced by more than 20 mm Hg in 32 cases, i. e. 91 per cent, in the erect position. Mean blood pressure was found reduced by more than 20 mm Hg in 34 of the 35 cases treated more than one month when measured in a recumbent or sitting position.

After an initial control period methyl dopa produced a diastolic blood pressure fall of 40 mm Hg or more in 3 women and 3 men in the erect position. Six women and 11 men had their diastolic blood pressure reduced by 20—39 mm Hg. In 9 women and 10 men less than 20 mm Hg reduction was observed. One woman was not examined in the standing position. In a sitting or recumbent position there was observed a diastolic blood pressure fall of 40 mm Hg or more in 3 women and 6 men. Nine women and 11 men had their diastolic blood pressure reduced by 20—39 mm Hg. In 7 women and 7 men less than 20 mm Hg reduction was observed (table I).

Working capacity has been recorded before and during treatment, and has improved in 12 patients (6 women and 6 men) or in about 1/3 of the patients treated more than one month (table II).

E.C.G. records before and during treatment were classified according to Rasmussen and Thingstad (12) and show a definit regress of pathological changes in 13 cases (table II).

Eye ground findings classified according to Keith et al. (5) show regress of pathological changes in 16 cases, including those with grade III and IV changes.

Serum creatinine was determined as a measure of renal function. At least in one woman (B. B.) the definite elevation of creatinine in blood decreased during treatment, but an increase of serum creatinine was observed in two cases during treatment.

Side effects

Only 7 patients all men have been completely free from side effects after the first week of treatment. The most frequent side effect has been drowsiness, which was observed in 17 cases, mostly but not exclusively in the first week of treatment (table III). Dizziness was found in 7 patients, in one of whom it was so troublesome that treatment had to be stopped. In 3 cases headache increased during treatment with methyl-dopa so that this treatment had to be stopped. Other side effects were: Disturbed vision, 1 case — impotence 2 cases — vomiting 1 case — constipation, 4 cases, — dry mouth, 1 case — breathlessness, 2 cases, — palpitations, 2 cases — auricular fibrillation 1 case — auricular flutter 1 case, — and fever 2 cases. In one of these fever reaction was reproduced 3 times with small doses of methyl-dopa. Leukocytosis but no eosinophilic reaction was found in this case.

In 13 patients treatment had to be discontinued and 8 of these patients had to stop treatment already in the first month often after few days of treatment only (table I, II and V).

A 49-year-old man (P. M.) died during the treatment period. He had considerable nephropathy and 4 mg/100 ml creatinine in serum. Previously he had tried ganglion blockers and other forms of therapy as well. Many years ago the had been sympathectomized. In this patient the effect on blood pressure and general state of health was good, but after 3 months of treatment he got heart arrhythmias while he stayed at home. He was then admitted to another hospital, where auricular flutter was diagnosed, which disappeared under quinidine therapy. After returning to his home, however he had a new attack and died within a few minutes. Autopsy was not performed. It is not probable that treatment with methyl-dopa had played any part in the fatal outcome in this case. In one other case, however auricular fibrillation was

The purpose of this report is to discuss our observations on the antihypertensive abilities of methyl-dopa (will be introduced as Aldomet® by Merck Sharp & Dohme) alone or in combination with saluric drugs

Material

During the period Nov 1960—Nov 1962 we have treated 43 patients with methyl-dopa, 19 women and 24 men. Average age was 56 years for men and 59 years for women. In 6 males and 2 females the treatment was stopped within one month on account of side effects. The remaining 35 patients have been treated for 430 months altogether i e. for an average of about 12 months Twenty two patients were given placebo tablets before treatment and 3 patients received placebo in an interval between two periods of therapy In 8 cases bed-rest alone was used during the control period, but 10 patients in whom the hypotensive treatment could not be stopped were given chlorthalidone (5) guanethidine (4) or Extr Rauwolfia (1) All patients received a normal hospital diet without salt restrictions. All patients were admitted to the medical department for evaluation before therapy was started. Their state was grouped according to Rasmussen's (11) system as follows Hypertensio arterialis laevis, 2 women and 8 men, — hypertensio arterialis gravis, 15 women and 14 men, — and hypertensio arterialis maligna, 2 women and ? men. Nephrogenic hypertension could not be excluded in 12 patients, 5 women and 7 men. One patient had low serum potassium values before treatment was started, 3,3 mEq/l After administration of potassium chloride per os, 3 g/24 hours for 8 days, the potassium values increased to 4 4 mEq/l Thus there probably was not true or primary hyperaldosteronism.

The concentration of cathecholamines in the urine was normal before therapy was started in all cases except three In one of these the urinary excretion of epinephrine was increased to 43 mg during 24 hours. Methyl dopa was ineffective in this patient. In another case the urinary excretion of nor epinephrine during 24 hours attained 64 mg Here methyl-dopa had a good effect. The third patient with an urinary epinephrine excretion of 350 mg and excretion of norepinephrine during 24 hours up to 690 mg was operated on but no phenochromocytoma could be found This patient suffered from palpitations and tinnitus during treatment with methyl-dopa

In all other cases the diagnosis of essential or primary hypertension was made. In one case there was a temporary blood pressure reduction following placebo. Otherwise increased blood pressure persisted during initial placebo treatment, which was generally combined with bed-rest.

Methods

Methyl-dopa was given alone to 8 cases, but only to 3 of the 35 patients treated for more than one month. In about 70 % of the material (17 men and 13 women) the drug was given in addition to rather small doses of hydrochlorothiazid — about 25 mg a day In 5 cases chlorthalidone 50 mg every other day was administered, and in 3 of these cases guanethidine had to be given in addition.

The treatment was generally inhibited with placebo for some days, whereafter methyl-dopa 250 mg × 3 daily or more was given. Maximum average dose was 1 000 mg daily Only in one case was 2,000 mg used. After some weeks the dose was often reduced, the average maintenance dose being between 600 and 900 mg/day divided into 3 or 4 separate doses.

Results

Blood pressure reduction

In the erect position normotensiveness (i. e blood pressure 150/90 mm Hg or below) was obtained during treatment in 10 cases or about 45 per cent but only in 3 cases was normotensiveness a constant finding in every control measurement. The average of the last three mean blood pressures (mean blood pressure = diastolic pressure + 1/3 pulse pressure) during treatment has been compared to the average of the last three mean blood pressures before treatment Mean blood

pressure was found reduced by more than 20 mm Hg in 3? cases, i. e. 91 per cent, in the erect position. Mean blood pressure was found reduced by more than 20 mm Hg in 34 of the 33 cases treated more than one month when measured in recumbent or sitting position

After an initial control period methyl dopa produced a diastolic blood pressure fall of 40 mm Hg or more in 3 women and 3 men in the erect position. Six women and 11 men had their diastolic blood pressure reduced by 20—39 mm Hg In 9 women and 10 men less than 20 mm Hg reduction was observed One woman was not examined in the standing position. In a sitting or ecumbent position there was observed a diastolic blood pressure fall of 40 mm Hg or more in 3 women and 6 men. Nine women and 11 men had their diastolic blood pressure reduced by 20—39 mm Hg. In 7 women and 7 men less than 20 mm Hg reduction was observed (table I)

Working capacity has been recorded before and during treatment, and has improved in 12 patients (6 women and 6 men) or in about 1/3 of the patients treated more than one month (table II)

E.C.G records before and during treatment were classified according to Rasmussen and Thingstad (12) and show a definite regress of pathological changes in 13 cases (table II)

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Serum creatinine was determined as a measure of renal function. At least in one woman (B. B.) the definite elevation of creatinine *in blood* decreased during treatment, but n increase of serum creatinine was observed in two cases during treatment.

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Only 7 patients, all men have been completely free from side effects after the first week of treatment. The most frequent side effect has been drowsiness, which was observed in 17 cases, mostly but not exclusively in the first week of treatment (table III) Dizziness was found in 7 patients in one of whom it was so troublesome that treatment had to be stopped In 3 cases headache increased during treatment with methyl-dopa so that this treatment had to be stopped Other side effects were Disturbed vision 1 case — impotence 2 cases, — omiting 1 case — constipation 4 cases, — dry mouth 1 case — breathlessness, 2 cases, — palpitations, 2 cases, — auricular fibrillation 1 case — auricular flutter 1 case — and fever 2 cases. In one of these fever reaction was reproduced 3 times with small doses of methyl-dopa. Leukocytosis but no eosinophilic reaction was found in this case.

In 13 patients treatment had to be discontinued and 8 of these patients had to stop treatment already in the first month, often after few days of treatment only (table I II and V)

A 49-year-old man (P M) died during the treatment period H had considerable nephropathy and 4 mg/100 ml creatinine in serum. Previously he had tried ganglion blockers and other forms of therapy as well. Many years ago the had been sympathectomized. In this patient the effect on blood pressure and general state of health was good, but after 3 months of treatment he got heart arrhythmia while he stayed at home H was then admitted to another hospital, where uricular flutter was diagnosed, which disappeared under quinidine therapy After returning to his home, however he had a new attack and died within a few minutes. Autopsy was not performed. It is not probable that treatment with methyl-dopa had played any part in the fatal outcome in this case. In one other case however uricular fibrillation was

Table 1 Reduction of blood pressure by methyl-dopa

Pat.	Age (yrs)	Average blood pressures (mm Hg)					Change		Lowest B. P during treatment	Other drugs
		Control (placebo) week		Lowest B. P during control	Final week of drug					
		Recumbent	Standing		Recumbent	Standing	Recumbent	Standing		
Women (19)										
Hypertensio labilis										
A. K.	62	210/120	180/130	180/120	195/115	180/125	−15/−05	0/−05	150/100	Hydrochlorothiazide
G. S.	54	200/120	200/120	200/100	200/90	190/100	0/−30	−10/−20	140/90	Hydrochlorothiazide
Hypertensio gravis										
S. A.	61	200/120	180/120	180/120	170/90	170/90	−30/−30	−10/−30	170/90	Hydrochlorothiazid
E. B.	59	240/140	220/140	190/120	190/125	190/130	−50/−15	−30/−10	120/90	Hydrochlorothiazide
E. C.	64	280/140	240/130	230 120	260/135	230 130	−20/−05	−10/0	—	None
A. E.	63	270/130	240/130	200/120	160/100	160/100	−110/−30	−80/−30	180/100	Hydrochlorothiazide
R. E.	59	280/180	220/130	200/120	180/120	180/130	100/−60	−40/0	160/110	Hydrochlorothiazide
A. Go.	72	280/150	240/125	40/120	200/120	190/125	−80/−30	−50/0	150/90	Chlorthalid + guanethidine
A. Gr	59	250/115	200/105	180/110	210/110	220/115	−40/−05	+20/+10	140/90	Hydrochlorothiazide
D. H.	62	180/120	180/130	180/120	160/105	170/120	−20/−15	−10/−10	150/100	Hydrochlorothiazide
E. S. H	41	260/140	—	180/100	180/100	—	−80/−40	—	180/100	None
G. H.	64	200/120	220/140	180/100	160/90	165/100	−40/−30	−55/−40	140/90	Hydrochlorothiazide
K. H.	53	200/100	200 120	170/100	160/100	150/90	−40/0	−50/−30	150/90	Hydrochlorothiazide
A. J	68	220/130	220/130	190/120	170/100	180/105	−50/−30	−40/−25	150/90	Chlorthalidone
E. J	67	220/120	225/125	180/100	180/100	180/110	−40/−20	−45/−15	140/80	Hydrochlorothiazid
K. L.	56	220/140	190/140	200/115	170/100	170/100	−30/−30	−20/−40	130/80	Chlorthalidone
M. P	4	220/120	210/120	140/80	180/100	160/100	−40/−20	−50/−20	160/100	Hydrochlorothiazide
Hypertensio maligna										
B. B.	51	260/155	280/170	240/130	170/115	170/110	−90/−40	110/−60	170/100	Chlorthalid. + guanethidine
E. M.	45	190/120	170/140	190/120	200/120	190/130	+10/0	+10/−10	170/105	Hydrochlorothiazide
Average B. P change for group							−45/−23	−30/−18		

Men (24)										
Hypertension benign										
A. B.	63	210/130	210/140	180/105	160/110	180/110	−30/−20	−50/−30	160/110	Hydrochlorothiazide
R. D.	53	230/130	180/120	170/115	160/100	160/105	−70/−30	−20/−15	130/100	None
W. D.	42	205/120	180/150	160/100	140/100	140/105	−65/−20	−40/−25	150/90	Hydrochlorothiazide
T. G.	58	230/120	170/120	150/110	140/85	180/110	−60/−25	+10/−10	150/100	Hydrochlorothiazide
H. Hau.	44	180/120	170/115	140/110	160/120	145/105	−20/0	−25/−10	125/105	None
O. K.	51	250/140	230/120	200/130	190/100	180/120	−60/−40	50/0	130/80	Hydrochlorothiazide
A. R.	64	210/120	200/120	160/110	150/100	155/110	−60/−20	−45/−10	130/80	Chlorthalid. + guanethidine
W. So.	59	210/110	180/110	190/100	170/100	170/100	−40/−10	10/−10	160/100	Hydrochlorothiazide
Hypertension grave										
A. G.	73	220/130	240/130	220/110	230/140	180/130	0/+10	−60/−20	—	None
H. H.	40	230/160	200/140	—	190/120	160/110	−60/−40	−40/−30	—	None
R. H.	50	190/120	180/110	180/100	165/110	155/110	−25/−10	−25/0	145/100	None
H. He.	51	200/130	200/140	140/110	160/100	170/105	−40/−30	−50/−35	130/90	Hydrochlorothiazide
J. J.	67	210/140	250/140	160/110	170/110	180/100	−40/−30	−70/−40	180/100	Hydrochlorothiazide
An. M.	61	210/110	200/120	200/110	180/90	185/100	−30/−20	−15/−20	170/90	Hydrochlorothiazide
E. N.	49	220/130	220/120	180/100	150/100	140/100	−70/−30	80/−20	130/80	Hydrochlorothiazide
W. P.	61	240/130	170/125	170/125	200/100	170/90	−40/−30	0/−35	—	None
O. R.	52	260/160	210/130	200/125	160/100	110/80	100/−60	−90/−50	110/80	Hydrochlorothiazide
W. Sv.	47	200/130	200/140	210/110	150/80	120/80	−70/−50	−90/−10	100/80	Hydrochlorothiazide
A. S.	54	180/120	170/110	150/110	170/105	160/110	−10/−15	−10/0	160/95	Hydrochlorothiazide
K. W.	45	150/110	150/120	140/90	170/110	160/110	+20/0	+10/−10	160/110	Hydrochlorothiazide
T. W.	57	270/130	220/150	220/130	160/90	155/100	110/−40	−65/−30	—	Hydrochlorothiazide
L. W.	66	220/140	210/140	190/110	180/110	170/105	−40/−30	−40/−35	140/100	Hydrochlorothiazide
Hypertension maligna										
E. E.	58	250/180	200/150	220/130	200/130	160/120	−50/−50	−40/−30	160/120	Hydrochlorothiazide
P. M. *	49	250/150	250/170	230/130	200/140	220/160	50/−10	−30/−10	—	Hydrochlorothiazide
					Average B. P. change for group		−45/−25	−38/−22		

Treatment stopped before one month. Nephrogenic hypertension not excluded. Treatment stopped later

Table II Other results

Pat.	Age (yrs)	Working capacity		ECG (Ref. 12)		Eye-ground findings (Ref. 5)		Creatinine in blood (mg %)		Serum potassium (mEq/l)	
		Before	After	Before	After	Before	After	Before	After	Before	After
Women (19)											
Hypertensio lucus											
A. K.	62	Partial	Full	II	Normal	II	I	1.1	1.2	5.3	4.8
G. S.	54	Partial	Partial	Normal	Normal	I	I	1.1	1.3	4.5	5.1
Hypertensio gravis											
S. A.	61	Partial	Partial	I	I	II	I	1.3	1.3	4.4	4.4
E. B.	58	Partial	Partial	III	III	II	II	1.2	1.4	4.2	4.8
E. C.	64	Partial	—	III	—	III	—	1.1	—	4.7	—
A. E.	63	Unfit	Full	II	I	II	I	0.9	1.0	5.3	4.4
R. E.	59	Unfit	Unfit	I	I	II	II	1.6	1.7	4.5	4.4
A. Go.	72	Partial	Partial	II	III	III	I	1.4	1.1	4.0	3.6
A. G.	59	Partial	Full	II	II	II	I	1.1	1.1	4.4	4.0
D. H.	62	Partial	Partial	I	Normal	II	I	0.9	0.9	5.3	4.4
E. S. H.	41	Unfit	—	I	—	I	—	1.1	—	4.5	—
C. H.	64	Partial	Full	II	I	II	I	1.1	1.1	4.2	3.9
K. H.	53	Partial	Partial	I	I	I	I	1.3	0.9	4.0	3.7
A. J.	68	Partial	Full	II	I	II	II	1.3	1.2	3.6	3.9
E. J.	67	Partial	Partial	III	II	II	II	1.2	1.1	4.2	4.0
K. L.	56	Partial	Full	IV	III	II	II	1.2	1.1	4.6	5.2
M. P.	74	Partial	Partial	I	Normal	II	II	1.2	1.0	4.4	4.4
Hypertensio maligna											
B. R.	51	Unfit	Partial	III	III	IV	II	3.0	1.7	4.3	3.7
E. M.	45	Partial	Partial	III	II	IV	II	1.2	1.3	4.3	3.4

Men (24)											
Hypertension lata											
A. B.	63	Unfit	Unfit	Normal	Normal	II	I	1.2	1.4	5.1	4.5
R. D.	53	Full	Full	I	Normal	II	I	1.8	1.5	5.3	3.2
W. D.	42	Partial	Full	Normal	Normal	Normal	Normal	1.4	1.0	4.3	4.4
T. G.	56	Partial	Full	I	I	Normal	I	1.1	1.0	4.0	4.0
H. Han.	44	Partial	—	Normal		I		1.5	—	4.0	—
O. K.	51	Partial	Full	I	I	I	I	1.2	1.5	4.8	4.4
A. R.	64	Unfit	Unfit	II	II	II	II	1.9	1.8	4.4	4.4
W. B.	59	Partial	Partial	II	III	III	II	1.8	1.8	4.0	4.0
Hypertension gravis											
A. G.	73	Unfit	—	III		III	—	1.9	—	5.0	—
H. H.	49	Partial	Unfit	I	Flat rr	II	I	4.1	4.2	5.0	—
R. H.	50	Full	—	II		III	—	1.1	—	4.6	—
H. He.	51	Full	Full	II	I	I	I	2.2	1.2	3.7	3.9
J. J.	67	Unfit	Unfit	III	III	II	II	1.6	2.0	4.7	4.2
An. M.	61	Full	Full	I	Normal	II	I	1.2	1.1	5.1	3.8
E. N.	49	Partial	Full	II	I	I	Normal	1.2	1.2	4.4	4.9
W. F.	61	Unfit	—	III	—	III	—	2.3	—	—	—
O. R.	52	Unfit	Partial	III	III	II	II	2.4	2.8	4.8	—
W. S.	47	Full	—	III		I	—	1.3	—	4.8	—
A. S.	54	Partial	Partial	I	I	I	I	1.1	1.3	4.1	3.8
K. W.	45	Partial	Full	I	I	II	II	1.8	1.5	4.1	3.4
T. W.	57	Full	Full	II	II	I	I	1.5	1.2	4.8	4.4
L. W.	66	Partial	Partial	II	II	II	II	1.0	1.0	5.4	4.6
Hypertension maligna											
E. E.	58	Unfit	Unfit	II	II	II	I	2.8	3.7	4.3	3.7
F. M.	49	Partial	—	III		III	—	2.3	—	3.6	—

Treatment stopped before one month.

Table III Side effects

	Women	Men	Total	Discontinued	
				Total	Before 1 month
Drowsiness	10	7	17	–	–
Dizziness	5	2	7	1	–
Headache	1	3	4	3	1
Constipation	1	3	4	–	–
Fever	–	2	2	2	2
Disturbed vision	–	1	1	1	1
Impotence	–	2	2	–	–
Breathlessness	1	1	2	1	1
Dry mouth	1	–	1	–	–
Vomiting	1	–	1	1	1
Auricular flutter	–	1	1	1 (more)	–
Auricular fibrillation	–	1	1	1	1
Palpitation	–	2	2	2 (more)	1
No side effect	–	7	7	–	–
			Total	15	8

produced and methyl-dopa stopped after one single day of treatment

A 45-year-old woman (E. M.) died from cerebral attack, presumably cerebral haemorrhage or thrombosis, a few days after treatment was stopped due to fatigue and palpitations. She had a two-sided homonomous hemianopsia, which occurred suddenly and a week later she died at home. No post mortem was carried out. This unfavourable result cannot be due to the alpha-methyl-dopa treatment, which was discontinued before the cerebral complication occurred.

Therapeutic response

Grading of the therapeutic response was done as follows An excellent response was considered to have been achieved when the diastolic pressure was consistently 95 mm Hg or less, and the side effects were minimal or absent. Good effect was present when the diastolic pressure was consistently between 95 and 110 mm Hg and side effects were no more than moderate, and a fair result was achieved when the diastolic pressure was above 110 mm Hg but showed a significant reduction (20 mm Hg or greater), or when the diastolic pressure was sometimes reduced to levels below 110 mm Hg, and when side effects were no more than moderate. Inadequate effect was considered to have occurred when the diastolic pressure was sometimes reduced but never to below 105 mm Hg or when side effects were severe.

An excellent effect on blood pressure and general state of health was observed in 7 cases, 3 women and 4 men A good effect was obtained in 18 cases (7 women and 11 men) fair results in 11 patients (5 women and 6 men) and an inadequate effect in 7 patients (4 women and 3 men)

Discussion and conclusions

The sedative effect of methyl-dopa cannot completely explain the methyl-dopa mechanism. In some cases there was an extraordinary effect both on blood pressure and on the patient's general state

of health. This gave us the impression that we had "hit the target" in these cases, this being patients who had tried other therapy without adequate effect. It is desirable that a test be found that can help to decide which patients are suitable for methyl-dopa therapy. Determinations of cathecholamine in the urine have given us little help in predicting the effect of the treatment. Theoretically one would believe that patients with increased cathecholamine urinary excretion and without phaeochromocytoma or with a questionable increase of cathecholamine excretion in the urine should be best suited for treatment with methyl-dopa.

Methyl-dopa might well also influence *the production of other pressor amines* by depressing their decarboxylation. The importance of serotonin in certain forms of hypertension is under discussion but we still know little about this. The effect of methyl-dopa in reducing blood pressure may also depend on other mechanisms, still unknown.

One may feel that our therapeutic results, 25 out of 43 patients with adequate effect, are not particularly good. However considering that most of the patients had tried other treatments before, without success, we suggest that the results are satisfactory. Side effects appeared early but have not been too troublesome. Positive tolerance for methyl-dopa was not observed during 2 years use. Therapy has not been given to patients with impaired liver function. We have not observed any case where treatment has led to liver impairment, measured by the usual liver function tests, including transaminase determinations (SGPT) which have been done regularly. We have tried to combine methyl-dopa and guanethidine, which, among other actions, antagonized constipation tendencies. The combination of methyl-dopa and saluretic has proved very valuable. Both chlorthalidone 50 mg every other day and hydrochlorothiazide 25 mg every day or every other day have, in our experiments, *increased effect* of *methyl-dopa* in reducing blood pressure. Severe hypopotassaemia was not observed during many months of this combined treatment. Increasing serum-creatinine was observed in a study of two cases during treatment. The significant hypotensive action particularly when quite small doses of a saluretic are given and in the lying position makes methyl-dopa a valuable new drug in the treatment of high blood pressure.

Summary

During a two-year period a total of 43 patients have been treated with methyl-dopa (19 women and 24 men). Average age was 56 years for men and 59 years for women. In 6 men and 2 women treatment was stopped within one month on account of side-effects. The remaining 35 patients have been treated for 430 months altogether i. e. an average treatment period of about 12 months. Placebo tablets have been given before treatment and in an interval between two periods of therapy. All patients were admitted to the medical department for evaluation before therapy was started. Their state was grouped as follow: Hypertensio arterialis laevis (2 women and 8 men), hypertensio arterialis gravis (15 women and 14 men) and hypertensio arterialis maligna (2 women and 2 men). Methyl-dopa was given alone in 8 cases. 30 patients (17 men and 13 women) were treated with methyl-dopa and hydrochlorothiazide. In 5 cases methyl-dopa was given in addition to chlor

thalidone. In 3 of these cases guanethidine had to be given in addition

Methyl-dopa was given in doses of 250 mg × 3 daily or more. Average maintenance dosage has been a little less than 1 g daily. An excellent effect on blood pressure and general state of health was observed in 7 cases and a good effect in 18 cases. Fair results were found in 11 patients and an inadequate effect in 7 patients.

Only 7 patients have been completely free from side effects. The most frequent side reaction has been drowsiness (17 patients). Considering that we have here treated patients who earlier failed to respond to other treatment the results seem to be satisfactory.

Positive increased tolerance has not been observed after two years medication with methyl-dopa.

In our studies we have not been able to find liver impairment in any case by the usual liver function tests. Combination of methyl-dopa and a saluretic has often proved valuable.

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Reversible Hypogammaglobulinaemia in Cyanocobalamin (B_{12}) Deficiency

By

C. K. V. van Dommelen, G. Slagboom, G. T. Meester and S. K. Wadman

In our ward, in 1955 a patient was seen who suffered both from pernicious anaemia in remission, and from serious persistent hypogammaglobulinaemia (14). Similar cases have been published by others (4, 7, 13, 16). In these cases the γ-globulin content failed to rise on treatment with cyanocobalamin. Larson et. al. (13) think that hypogammaglobulinaemia is the primary fault and that repeated infections of the intestine are a possible consequence, giving rise to malabsorption and cyanocobalamin deficiency.

Recent observations in our clinic suggest other possible explanations. In 1955 we saw a patient with untreated pernicious anaemia and moderate hypogammaglobulinaemia; both conditions responded to treatment with cyanocobalamin. Thus our attention was drawn to reversible hypogammaglobulinaemia in pernicious anaemia. From 1955 to 1960 several such patients were seen; since no similar cases were found in the literature we feel it justified to report our findings.

Material

Group A consisted of 49 patients aged from 40 to 90 years, with untreated cyanocobalamin deficiency. Forty-eight of them suffered from classical addisonian anaemia; none of them had any complicating disease known to give rise to high γ-globulin values (e. g. chronic infections, cirrhosis of the liver). The diagnosis was confirmed by the typical blood picture, gastric achylia, megaloblastic bone marrow, low cyanocobalamin content of the serum, typical response to treatment; results of Schilling test, when performed, were abnormal.

Pre-treatment haemoglobin levels ranged from 5 to 14.5 g/100 ml.

The 49th patient of this group was a woman, aged 36, who had cyanocobalamin deficiency with megaloblastic anaemia after resection of 150 cm of the terminal ileum because of fibroma; her stomach did secrete free hydrochloric acid.

Control group B consisted of 40 out-patients, in whom after thorough examination, diagnosis of irritable colon only could be made. The 40 were matched in age and sex to the first 40 patients with pernicious anaemia of our series. They served us as "normal controls".

Control group C consisted of 39 patients with iron deficiency anaemia, caused by

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Table I Frequency of γ-globulin levels (ammonium-sulphate turbidity test)

γ-globulin	Group A	Group B	Group C
	Cyanoco-balamin deficiency	Normal controls	Iron deficiency anaemia
0.4	2	1	—
0.5	6	—	2
0.6	5	—	2
0.7	3	2	4
0.8	8	3	[illegible]
0.9	11	3	6
1.0	3	1	3
1.1	8	5	3
1.2	1	3	3
1.3	—	7	4
1.4	1	6	—
1.5	—	4	3
1.6	1	2	4
1.7	—	—	1
1.8	—	1	2
1.9	—	—	—
2.0	—	2	—
Mean	0.84	1.24	1.20
S. D	0.25	0.35	0.37
Total no. of pat.	49	40	39

excessive menstrual blood loss, insufficient iron intake, aspirin abuse, bleeding peptic ulcer or hiatus hernia. None of them had shown massive bleeding recently Those in this group were likewise matched in age and sex to 39 patients of group A, chosen at random. Haemoglobin values varied from 4.5 to 9.5 g/100 ml.

Methods

γ-globulin was estimated by the ammonium sulphate turbidity test according to de la Huerga and Popper (10) as described by us in a former publication (5) Extinctions were read in a Beckman DU Spectrophotometer A calibration curve was obtained by comparison with free electrophoresis. γ-globulin values as determined by both methods are in good agreement. From a series of duplicates we calculated a coefficient of a correlation r = 0.91 (36 sera with a γ globulin content between 0.4 and 1.25 as determined by electrophoresis)

For electrophoresis sera were diluted with twice their volume of buffer solution (veronal-veronal Na, pH 8.5 ionic strength 0.1). Diluted sera were dialyzed during 48 hours at 0° in cellophane tubing against the same buffer solution Electrophoresis was carried out in a compact Tiselius apparatus model 38 Perkin Elmer Corporation (19) current 11 mA. Optical registration by the Longsworth Schlieren scanning method The amounts of serum protein fractions were obtained from the areas between the theoretical Gauss-curves and the baselines. The total protein concentration was determined by the micro-Kjeldahl method.

The standard deviation for both methods of determining γ-globulin (ammonium sulphate turbidity or electrophoresis plus micro-Kjeldahl procedure) was about 0.05 g/100 ml

Since 1955 in every patient with cyanocobalamin deficiency serum γ-globulin has been estimated by the ammonium sulphate turbidity test, before treatment.

In the first years of our investigation electrophoresis was performed only in sera which gave low ammonium sulphate turbidity readings. Later on, however electrophoresis was routinely carried out in all untreated cases of pernicious anaemia. Electrophoretic investigation was repeated in all instances after remission had been induced by treatment with cyanocobalamin. Intervals between two determinations in one patient varied from one month to 3 1/2 years the mean time was one year

Results

A Low γ-globulin values in cyanocobalamin deficiency

For statistical evaluation only the results of ammonium sulphate turbidity tests are available (the electrophoresis group being selected)

In normal controls (group B) and in persons with iron deficiency anaemia (group C) the mean values and standard deviations of serum γ-globulin content

Table II. Electrophoretic separation of serum proteins in cyanocobalamin deficiency before and after treatment. Haemoglobin and all protein values are expressed in g/100 ml

Pat. no.	Months elapsed between tests	Hb	Before						After					
			Total prot.	Alb.		α_2	β	γ	Total prot.	Alb.			β	γ
1	36	6.8	5.4	3.1	0.3	1.2	0.4	*0.4*	6.9	3.4	0.2	1.6	0.8	*0.95*
2	6	6.5	5.9	3.4	0.6	0.7	0.7	*0.5*	6.2	3.45	0.35	0.6	0.8	*1.0*
3	8	5.8	5.6	3.2	0.55	0.6	0.7	*0.55*	6.8	3.8	0.35	0.65	1.05	*0.95*
4	6	7.3	6.2	3.9	0.45	0.6	0.7	*0.55*	7.1	3.85	0.35	0.75	1.1	*0.85*
5	18	7.7	6.1	4.0	0.4	0.5	0.6	*0.6*	7.8	3.5	0.6	0.7	1.2	*1.0*
6	24	5.3	6.5	3.8	0.5	0.6	0.9	*0.7*	7.3	3.85	0.55	0.8	1.15	*0.95*
7	3	14.5	6.4	3.8	0.5	0.8	0.6	*0.7*	7.2	4.25	0.5	0.65	0.75	*1.05*
8	24	10.5	6.3	3.8	0.3	0.7	0.7	*0.8*	6.6	4.0	0.55	0.55	0.8	*0.7*
9	18	6.5	6.8	4.2	0.3	0.6	0.9	*0.8*	7.5	3.6	0.7	0.95	1.15	*1.1*
10	2	11.0	6.4	3.5	0.4	0.8	0.85	*0.85*	6.5	3.1	0.45	0.85	1.0	*1.1*
11	2	5.2	5.7	3.1	0.6	0.5	0.65	*0.85*	7.4	3.75	0.6	0.7	1.15	*1.05*
12	5	12.7	5.8	2.65	0.5	0.65	1.15	*0.85*	6.5	3.1	0.4	0.85	1.25	*0.75*
13	16	4.1	5.8	3.1	0.4	0.6	0.8	*0.9*	7.3	4.3	0.45	0.75	0.85	*0.95*
14	12	5.7	5.3	2.9	0.3	0.4	0.8	*0.9*	6.8	4.3	0.3	0.8	0.6	*0.8*
15	36	6.0	6.2	3.7	0.4	0.5	0.7	*0.9*	7.5	4.9	0.5	0.45	0.8	*0.85*
16	2	6.8	7.0	3.95	0.55	0.5	1.0	*1.0*	7.4	3.2	0.75	1.0	1.15	*1.25*
17	12	7.5	6.8	4.0	0.4	0.55	0.8	*1.05*	7.3	4.25	0.5	0.75	1.05	*1.15*
18	2	9.2	6.7	2.6	0.7	0.9	0.9	*1.2*	7.5	3.85	0.45	0.75	1.2	*1.25*
		Mean	6.2	3.5	0.45	0.65	0.75	0.8	7.1	3.8	0.5	0.8	1.0	1.0
		S. D.	0.45	0.30	0.12	0.13	0.17	*0.21*	0.42	0.48	0.16	0.15	0.20	*0.16*
9 normal controls		Mean	7.5	3.8	0.40	0.69	1.0	1.0	—	—	—	—	—	—
		S. D	0.4	0.2	0.13	0.16	0.2	0.17	—	—	—	—	—	—
Neill and Weaver's 16 patients with pernicious anaemia														
		Mean	6.14	3.52	0.30	0.55	0.53	1.30	7.22	4.34	0.23	0.47	0.93	1.24
		S. D	0.70	0.79	0.14	0.16	0.17	0.27	0.33	0.3	0.14	0.04	0.02	0.2

were equal (table I). In cyanocobalamin deficiency (group A) the mean γ-globulin content was much lower, the difference being statistically highly significant (t test, $p < 0.001$).

In 15 out of 49 patients (24 %) with cyanocobalamin deficiency subnormal γ-globulin was found (lower than 0.7 g/100 ml) whereas this was found in one of 40 healthy persons (2.5 %) and 4 of 39 cases of iron deficiency (11 %).

B. Rise in γ-globulin content during cyanocobalamin therapy

Results of repeated electrophoretic serum-protein determinations in 18 patients with cyanocobalamin deficiency before treatment and during remission, are shown in table II. There was a statistically significant rise in γ-globulin values: the mean value before treatment was 0.78 g/100 ml and during remission it was 0.98 g/100 ml ($p = 0.004$).

Table III Repeated electrophoretic separation of serum proteins

Pat. no.	Total protein	Alb.	α_1	α_2	β	γ	Time of electrophoresis
1	5.4	3.1	0.3	1.2	0.4	0.4	Untreated
	6.7	3 1	0 6	1.6	0.6	0.8	After 1 month
	7.5	4.2	0.3	1 7	0.7	0.6	After 24 months
	6.9	3.4	0.2	1.6	0.8	0.95	After 36 months
5	6.1	4.0	0.4	0.5	0.6	0.6	Untreated
	7 1	4.2	0.4	0.85	0.85	0.8	After 9 months
	7 0	3.5	0.6	0 7	1.2	1.0	After 18 months
8	6.3	3.8	0.3	0.7	0 7	0.8	Untreated
	6.5	4.2	0.4	0.65	0.7	0.55	After 12 months
	6.6	4.0	0.55	0.55	0.8	0.7	After 24 months
3	5.6	3.2	0.55	0.6	0.7	0.55	Untreated
	6.9	4.3	0.5	0.5	0.9	0.7	After 1 month
	6.8	3.8	0.35	0.65	1 05	0.95	After 8 months

In no instance were subnormal values seen during remission. In four patients electrophoretic determinations were repeated more than once in three of them the last gammaglobulin value was highest (table III)

It appears from table II that almost all patients showed a rise in γ-globulin content, not merely those who had low values before treatment. This rise may be considerable from 0 4 to 0 95 g/100 ml (pat. 1) from 0 5 to 1 0 (pat. 2) from 0 6 to 1 0 (pat. 5)

C Doubtful reliability of ammonium sulphate test in detecting hypogammaglobulinaemia

Though in most cases the ammonium sulphate test and electrophoresis gave the same result, ammonium sulphate readings of 0 7 masked hypogammaglobulinaemia in two instances in other cases subnormal ammonium sulphate values were not confirmed by electrophoresis.

D Other protein fractions

Data in table II show that before treatment mean levels of albumin, α_2-globulins and β-globulins were also low or even subnormal, and that they rose to normal after treatment. The rise in β-globulin was most constant, appearing in all but one patient. The rise in α_2 globulins was statistically significant (p = 0 003) the rise in albumin was not (p = 0 08) α-globulins were normal before treatment and did not change.

E Correlation between changes in haemoglobin content and various serum protein values

Correlation coefficients calculated between the following data in untreated cases were

a haemoglobin and γ-globulin + 0 125 b albumin and γ-globulin — 0 2 c. β-globulin and γ-globulin + 0 64 d. α_2-globulin and γ-globulin — 0.29 e. haemoglobin and albumin 0 £ albumin and α_2-globulin — 0.35

Only the difference from zero shown by the positive correlation between β- and γ-globulin contents was statistically significant (0 01 > p > 0 001) These calculations confirm the impression one

gets when looking through table II γ-globulin may be low even when haemoglobin (no. 7) or albumin (no. 5) is rather high.

Discussion

Data on γ-globulin values in untreated cyanocobalamin deficiency are scarce in the literature. Wuhrmann and Wunderley (24) state that the values are normal but that β-globulin and albumin are lowered. Riva (22) found a high γ-globulin content in 4 out of 5 patients. Neill and Weaver (20) studied protein fractions calculated by means of paper electrophoresis, in 30 patients with pernicious anaemia in relapse, and repeated the analysis after treatment in 16 of these. Total protein, albumin α_1- and (most striking) β-globulin contents were low before, and rose to normal after treatment, α_2-globulin did not change; these figures are almost equal to ours (table II). But in their cases γ-globulins were normal in relapse and did not change after treatment. This is a surprising difference from our data. It may be related to differences in the methods used. Neill and Weaver used filter-paper electrophoresis, we used free electrophoresis. But a crude method, the ammonium sulphate turbidity test, also gave low values in many of our patients — indeed by this method we detected the occurrence of hypogamma-globulinaemia in pernicious anaemia. Neill and Weaver observed their patients for no longer than 6 months, whereas in our cases the second electrophoresis was usually performed much later. But this does not explain why we found low pre-treatment levels, and even after two or three months a rise was seen (cases 7, 10 and 11).

Another point of difference between their and our cases may be that we excluded cases complicated by diseases that may cause high γ-globulin values. Neill and Weaver give only mean values for their whole group of patients. If they did include cases complicated by, for instance, chronic infections, this might have caused unduly high γ-globulin values. But one may ask nevertheless why their values did not rise after treatment.

As do Neill and Weaver, we think the observed fall in serum-protein values is caused by the deficiency in cyanocobalamin and not by the anaemia as such, because the mean level of γ-globulin was normal in iron-deficiency anaemia. This fall was not caused by some related but different deficiency since there was a rise after treatment with pure cyanocobalamin, even in the patient in whom the terminal part of the ileum had been excised.

A fall in plasma proteins may be caused by

a. decreased synthesis
b. increased catabolism in the tissues or in the gut after loss through an affected wall
c. increased plasma water
d. passage into extravascular spaces.

Simple dilution (c) cannot explain our data, because changes in fractions are on the whole not correlated. On the other hand, plasma dilution has been found in pernicious anaemia (9) so it might be a contributing factor. In view of the well-known atrophic changes of tongue and gastric mucosa, study of protein loss into the gut with the use of tracer techniques seems warranted. However, if loss into the gut were a major cause, one might expect that albumin levels would be relatively lower than γ-globulin values and that the levels of both fractions would

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Pat. no.	Total protein	Alb.	α_1	α_2	β	γ	Time of electrophoresis
1	5.4	3.1	0.5	1.2	0.4	0.4	Untreated
	6.7	3.1	0.6	1.6	0.6	0.8	After 1 month
	7.5	4.2	0.5	1 7	0.7	0.6	After 24 months
	6.9	3.4	0.2	1 6	0.8	0.95	After 36 months
5	6.1	4 0	0.4	0.5	0.6	0.6	Untreated
	7 1	4.2	0 4	0.85	0.85	0.8	After 9 months
	7.0	3.5	0.6	0.7	1.2	1 0	After 18 months
8	6.3	3.8	0.3	0.7	0.7	0.8	Untreated
	6.5	4.2	0 4	0.65	0 7	0.55	After 12 months
	6.6	4 0	0.55	0.55	0.8	0.7	After 24 months
3	5.6	3.2	0.55	0.6	0.7	0.55	Untreated
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Correlation coefficients calculated between the following data in untreated cases were

a haemoglobin and γ-globulin + 0 125 b albumin and γ-globulin — 0.2 c. β-globulin and γ-globulin + 0 64 d. α_2-globulin and γ-globulin — 0 29 e haemoglobin and albumin 0 [illegible] albumin and α -globulin — 0 35

Only the difference from zero shown by the positive correlation between β- and γ-globulin contents was statistically significant ($0.01 > p > 0.001$) These calculations confirm the impression one

gets when looking through table II γ-globulin may be low even when haemoglobin (no. 7) or albumin (no. 5) is rather high.

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Only the difference from zero shown by the positive correlation between β- and γ-globulin contents was statistically significant ($0.01 > p > 0.001$) These calculations confirm the impression one

it appears that the same holds true for involvement of liver cells and plasma cells. So hypogammaglobulinaemia may be the initial symptom of pernicious anaemia — as was more or less the case in our patient 7 — while in other cases γ-globulin levels may be normal.

One may ask if there is a relation between this reversible hypogammaglobulinaemia and the more serious cases of agammaglobulinaemia in pernicious anaemia. In these latter cases γ-globulin levels were very low though the patients had been injected with cyanocobalamin for years, so that haemoglobin was normal. One might postulate that, when cyanocobalamin deficiency develops in a patient suffering from chronic infection, e. g. purulent bronchitis, the plasma cell system might become totally and irreversibly damaged. Such irreversible damage to one cell system may occur in one other symptom of pernicious anaemia: the combined degeneration of the spinal cord but it is not known to occur in the other manifestations of the disease. Evidently this point remains uncertain.

Another question is, whether our observations have any practical implications. At this moment we feel justified only in advising to look for cyanocobalamin deficiency in cases of otherwise unexplained hypogammaglobulinaemia, because early diagnosis may save the spinal cord (and, hypothetically the plasma cell system?) Further investigations are needed to settle the role (if any) of cyanocobalamin in resistance to infections and response to vaccinations. And one might speculate about the use of a cyanocobalamin antagonist in the treatment of multiple myeloma (and hope that this antagonist would rather inhibit growth of myeloma cells than destroy nerve cells in the spinal cord.)

Summary

By the ammonium sulphate turbidity test, γ-globulin levels below 0.7 g/100 ml were found in 13 out of 49 patients with cyanocobalamin deficiency. By repeated electrophoretic examination this hypogammaglobulinaemia was shown to be real and reversible by treatment with cyanocobalamin. It is supposed that cyanocobalamin deficiency impairs function of the plasma cells and synthesis of γ-globulins.

A search for cyanocobalamin deficiency in unexplained hypogammaglobulinaemia is advised.

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be corrrelated neither situation occurred in our cases In pernicious anaemia there sometimes is increased excretion of amino acids in the urine (20) This suggests that decrease protein synthesis (a) or increased catabolism (b) may be the cause of the hypoproteinaemia.

How cyanocobalamin acts in metabolism is little known So far presence of a cyanocobalamin coenzyme has been proved only in one type of enzyme these are isomerases which aid in the metabolism of propionic and methylmalonic acids, both in protozoa (2) and in higher animals (8 12 15) But it is not known if and how this process is related to protein synthesis.

Experiments in which a direct role of vitamin B_{12} in protein synthesis seemed to be shown (23) were negative when repeated (1 6)

One reaction in protein synthesis is the transfer of one-carbon fragments ($-CH_3$, $=CH_2$, $\equiv CH$ $-CHNH$ $-CHO$ and $-CH_2OH$) This transfer is mediated by tetrahydrofolic acid Vitamin B_{12} probably aids somewhere in the conjugation between these one-carbon fragments and tetrahydrofolic acid (3) Though it has long been suspected that vitamin B_{1} has a role in the synthesis of nucleic acids, this could never be proved Recently however Luhby and Cooperman (17) claimed to have found in cyanocobalamin deficiency a specific block in the synthesis of inosinic acid and therefore of nucleic acids. Such a block would indirectly inhibit protein synthesis it would be in agreement with our data

In the bone marrow not only young red cells but also white blood cells and platelets need cyanocobalamin for their growth It is tempting to suppose that plasma-cells also need it If this were the case decreased synthesis of γ- β_{2A} and β_{2M}-globulins could be expected in cyanocobalamin deficiency and hypogammaglobulinaemia would be explained this is consistent with the correlation between γ- and β-globulin levels, as observed by us.

Other facts suggest that plasma cells do need cyanocobalamin Mandema (18) observed low serum cyanocobalamin levels in cases of myeloma he supposes that cyanocobalamin is consumed by the great number of (abnormal) plasma cells he cites authors who saw megaloblastic changes in erythropoiesis in myeloma. In our clinic a patient was seen in whom typical cyanocobalamin deficiency with megaloblastic anaemia seemed to be caused by a new growth of atypical plasma cells with macroglobulinaemia (21)

Conclusions

From the foregoing we conclude that to five "classical signs of cyanocobalamin deficiency anaemia, leukopenia, thrombopenia glossitis, and combined degeneration of the spinal cord should be added the low values of albumin and α and β-globulins described by Neill and Weaver and the hypogammaglobulinaemia of our cases. In the five classical signs five different cell types are involved. The hypalbuminaemia of Neill and Weaver points to disturbed protein synthesis in the liver cells, and our hypogammaglobulinaemia to disturbance of plasma-cell function The classical signs are not closely correlated each of them may be the presenting symptom of pernicious anaemia and on the other hand involvement of one cell system may be lacking in an otherwise full-blown picture of the disease. From the correlation coefficients between protein fractions and haemoglobin values calculated by us,

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From one of the Medical Departments of the Municipal Hospital, The Hague, Holland

Sodium Economy in the Proximal and Distal Parts of the Nephron Studied in Patients with Diabetes Insipidus

Their Estimation under Normal Conditions and after Salt Restriction, Chlorothiazide and a Mercurial Diuretic

By

P. S. Blom, L. Roos and H. A. Schoevelt

Since Hilger et al. (5) have shown that the collecting ducts of the kidney partake in active sodium reabsorption, it may be said that nearly the whole length of the renal tubule acts as a sodium-conserving system, the only exception being the descending limb of the loop of Henle. There may however be important differences in the regulatory forces governing sodium conservation and the quantities handled at different tubular levels.

It is clear that stop-flow experiments cannot produce quantitative data or show the effect of a single variable under physiological circumstances. Heinemann et al. (4) have drawn attention to the fact that the formation of "free water" (water in surplus of an isosmotic requirement) is function of the distal parts of the tubule since proximal tubular urine has been shown to be isosmotic under widely varying circumstances (3, 12, 13, 14). They used free water formation as a parameter of distal tubular function under the influence of different diuretics. To exclude the effect of A.D.H. they had to overhydrate their subjects, except for an occasional patient with diabetes insipidus. The use of patients with diabetes insipidus is preferable since their condition makes a free choice of the state of hydration possible. However more explicit information on distal tubular sodium reabsorption can be obtained as follows.

Since the osmolarity of tubular urine at the far end of the proximal tubule is identical with plasma osmolarity

$$P_{osm} \times V - U_{osm} \times V = T_{osm\,dist} \qquad (1)$$

(where P_{osm} and U_{osm} designate osmolarity of plasma and urine respectively and V is the flow of tubular urine entering the distal segment, while V is the ultimate urine-flow. $T_{osm\,dist}$ indicates osmolar reabsorption in the distal part of the nephron)

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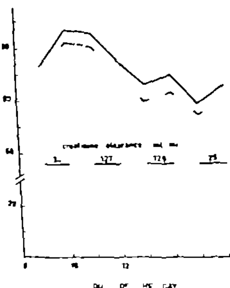


Fig. 1 Case 1 Diurnal variation of distal sodium inflow and reabsorption (blank experiment) Inflow into distal segment and distal reabsorption have been calculated from experimental data and have been transformed into percentages of mean also I this case the mean inflow value for 8—12 clock (1946 μEq/min.) was used as standard for transformation of both inflow (——) and reabsorption ( ) into percentages 100 = 1,946 μEq min
Na-intake (8 a.m.—4 p.m.) 68.5 mEq.
Na-excretion (8 m—4 p m.) 48.0 mEq
Endogenous creatinine clearance values were measured over the hours as indicated.

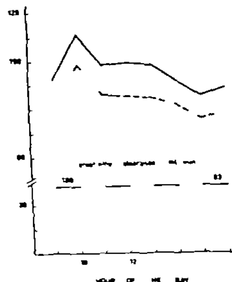


Fig. 2. Case 2. Diurnal ariation f distal sodium inflow and reabsorption (blank experiment). Cf. legend fig. 1
Na inflow (——) Na reabsorption ( )
Standard for transformation into percentage values is mean Na inflow from 8 a.m to 1 p.m. (1,289 μEq/min. = 100)
Na-intake (8 a.m.—4 p.m.) 68.5 mEq
Na-excretion (8 a.m.—4 p.m) 71.4 mEq.
Complete data of this experiment are given in table I as an example.

All chemical determinations were done by standard methods. Plasma creatinine was determined mostly by the method of de Vries and van Daatselaar In part of the experiments no correction for chromogens was made however

Cas reports

Case 1 Woman, age 30 years, weight 63 kg. Diabetes insipidus since Aug. 1959 Experiments done in period from Nov 1959 to March 1961 Cause of disease still unknown. Absence of A.D.H. established unequivocally by Hickey-Carter test and supervised period of thirsting Several parameters of anterior lobe function Normal (F.S.H. excretion, insulin sensitivity) Creatinine clearance 127—134 ml/min.

Urine volumes without treatment 10—14 1/24 hrs.

Case 2. Woman, age 35 years, weight 63 kg. Diabetes insipidus since June 1960 after severe head-injury Experiments done in period from Oct. 1960 to March 1961 Absence of A.D.H. established by Hickey-Carter test. Parameters of anterior lobe function Normal (F.S.H. *excretion, insulin sensitivity*) Creatinine clearance (not corrected for chromogens): 108—114 ml/min. Urine volumes untreated 10—14 1/24 hrs.

When A.D.H. production is suppressed or permanently absent as in diabetes insipidus, water reabsorption in the distal tubules (including the collecting ducts) is considered to be negligible; therefore $V_1 = V_2$ (= V) and

$$V(P_{osm} - U_{osm}) = T_{osm.dist.} \quad (2)$$

$T_{osm.dist.}$ can thus be measured easily. It is known that this osmolar reabsorption in the distal part of the nephron consists largely of reabsorbed sodium and accompanying anion. Distal sodium reabsorption ($T_{Na.dist.}$) must be somewhat larger than indicated by $T_{osm.dist.}$ because this value gives an over-all balance figure for distal osmolar transfers in which the distal production of NH_4 and K may have an influence on the negative side. Assuming complete proximal reabsorption of filtered K and subsequent distal excretion of the K found in the excreted urine, it may be said that

$$T_{osm.dist.} = 2(T_{Na.dist.} - U_{NH_4}V - U_K V) \quad (3)$$

Since the excretion of NH_4 on a normal diet will not exceed 50 μEq/min, which is less than 10% of distal sodium reabsorption, we have omitted this measurement. So (3) is simplified to

$$T_{osm.dist.} = 2(T_{Na.dist.} - U_K V) \quad (4)$$

From (2) and (4) we derive that in case of diabetes insipidus

$$T_{Na.dist.} = \frac{V(P_{osm} - U_{osm})}{2} + U_K V \quad (5)$$

$U_{Na}V$ is the sodium which escaped reabsorption in the distal segment. If we call the inflow of sodium into this segment $Infl_{Na.dist.}$ it may be said that

$$Infl_{Na.dist.} = T_{Na.dist.} + U_{Na}V \quad (6)$$

We can thus measure $Infl_{Na.dist.}$ which is an important variable because distal reabsorption ($T_{Na.dist.}$) can only be judged in the light of proper knowledge of this inflow which at the same time offers some parameter of the combined actions of glomerulus and proximal tubule regarding sodium economy.

A more extensive discussion of the basic presumptions underlying our method of calculation and presentation and of the inaccuracies resulting from them is given under "Further comments".

Methods

Experiments were done in two young women suffering from diabetes insipidus. Pitressin (tannate) injections were stopped at least 72 hours before the experiments. Two different experimental designs were followed.

In the long term experiments, lasting several days, the subjects were kept on a rigid dietary scheme either of low sodium content or with a fixed quantity of sodium-chloride added in weighed amounts. For keeping up a fairly constant state of hydration in relation to the sodium balance we relied upon the regulatory function of thirst. The patients had constant and convenient access to water.

In the short term experiments the patient was strictly kept in a constant state of hydration from 8 a.m. until 3 or 4 p.m. by the drinking every hour of a quantity of water equal to the quantity of urine produced in the preceding hour. We started with one hour of free choice of water intake. This scheme makes no allowance for insensible losses of water but duration of the experiment is only 8 hours. To compensate sodium losses in the short-term experiments, sodium chloride was given every hour in a fixed quantity estimated to equal in 7 or 8 doses the total loss during the experiment. Quantities of intake and output of sodium during the experiment are shown in the results. The patient started the experiment after overnight fasting but received either 10 g of glucose or 2 dry biscuits hourly thereafter to prevent undesirable side-effects of hunger and increasing acidity of the urine.

Osmolarity of the plasma and urine were calculated from concentrations of Na, K and urea according to

$$\text{Osmolarity (mOsm/l)} = 2 \times Na\,(mEq/l) + 2 \times K\,(mEq/l) + \frac{Urea}{60}(mg/l)$$

Variations in plasma osmolarity did not exceed 5 mOsm/l in repeated determinations during short term experimental or control periods, so mean values could be used in calculations within one experimental and one control period.

Calculations of $T_{Na.dist.}$ and $Infl_{Na.dist.}$ were done according to formulas (5) and (6).

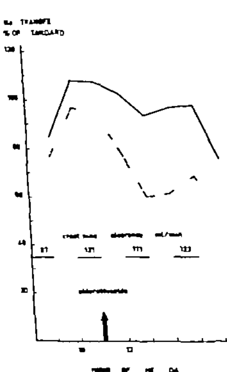


Fig. 3. Case 1 Acute effect of chlorothiazide on distal sodium inflow and reabsorption. Cf. legend fig. 1.
Na inflow (——) Na reabsorption ( ).
Standard for transformation into percentage values mean Na inflow from 8 a.m. to 11 a.m. (1,630 μEq/min. = 100).
At 11 a.m. 0.5 g of chlorothiazide was given orally
Na intake (8 a.m.—4 p.m.) 112 mEq
Na excretion (8 a.m.—4 p.m.) 150 mEq.

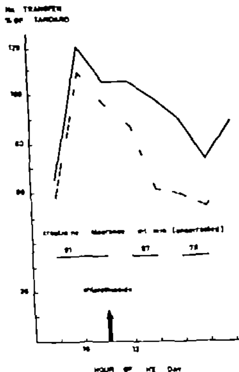


Fig. 4. Case 2. Acute effect of chlorothiazide on distal sodium inflow and reabsorption. Cf. legend fig. 1
Na inflow (——) Na reabsorption ( ).
Standard for transformation into percentage values is mean Na inflow from 8 a.m. to 11 a.m. (1 138 μEq/min. = 100)
At 11 a.m. 0.5 g of chlorothiazide was given orally
Na intake (8 a.m.—4 p.m.) 141.5 mEq
Na excretion (8 a.m.—4 p.m.) 118 7 mEq.

somewhat higher than the calculated values, but always by the same (absolute) amount (see formulas 5) and 6)) During both the short-term and long-term experiments the osmolar concentration of the final urine increases about 1 1/2 to 3 times. If this is followed, as Orloff suggests, by a better conservation of water within the tubular lumen, the left hand parts of some of our diagrams (control periods) are at a lower level than they should be in comparison with the right hand parts, showing the experimental periods. The conclusions are not invalidated however by this correction.

Short-term experiments

All experiments were done with the patients walking around or sitting in an easy chair

The values for sodium inflow and extraction during the first hour are throughout considerably lower than in the following hours of the control period. No explanation of this finding can be offered.

Table I Blank experiment case 2 Cf fig 2 All experimental results in urine were obtained in one hour portions and tabulated as mean per minute

Period	Observed excretion values in urine/min						Plasma-osmolarity[1] (μOsm/ml)	Derived sodium transfer values per min			
	V (ml)	Na (μEq)	K (μEq)	Urea (μOsm)	Sol. excr (μOsm)	Free H_2O (ml)		Na inflow dist. (μEq)	Na reabs. dist. (μEq)	Na inflow dist. (% of standard)	Na reabs. dist. (% of standard)
a. m.											
8–9	8.5	13	85	255	695	6.5		1 198	1 063	92.9	82.4
9–10	10	158	91	263	761	7.5	314	1 428	1,270	110.7	93.3
10–11	8.9	161	75	233	705	6.6		1,271	1 110	98.6	86.1
11–12	9	183	76	243	761	6.5	316	1,282	1,099	99.4	85.2
p. m.											
12–1	8.9	171	71	243	727	6.5		1,266	1 095	98.2	84.9
1–2	8.3	126	48	225	573	6.4	310	1 182	1 056	91.6	81.9
2–3	7 7	123	38	201	523	6.0		1 100	977	85.3	75.7
3–4	8	130	41	211	555	6.2	307	1 141	1 011	88.5	78.4

Solutes excretion (μOsm) calculated as 2 × Na (μEq) + 2 × K (μEq) + urea (μOsm)

Free H_2O calculated as $V - \frac{\mu Osm/min\ urine}{\mu Osm/ml\ plasma}$

Plasma osmolarity μOsm/ml calculated as 2 × Na (μEq/ml) + 2 × K (μEq/ml) + $\frac{urea\ (\mu g/ml)}{60}$

Plasma osmolarity was considered consta t at its mean value (312 μOsm/ml) for the whole experiment.

The estimation of the distal sodium reabsorption was by means of formula 5) (page 202)

$Inflow_{Na\ dist.} = T_{Na\ dist.} + U_{Na}V$

The mean of the first 5 hours (1,289 μEq min) was used as a standard for conversion of experimental results in percentage values.

Results and comments

Basic notation of experimental data was made in units (μEq or ml) per minute calculated as a mean value over the experimental period of urine collection All data on sodium transfer are presented in the diagrams as a percentage of mean $Infl_{Na\ dist.}$ during control hours or days. This way of presentation shows fluctuations in the data of control and experimental periods free of distortion Comparison of distal tubular function with the simultaneous inflow values can be done by comparing ordinate length instead of reading percentage values.

At this point we should mention that our results may have been affected to some extent by a loss of water in distal or collecting tubules True values of distal sodium reabsorption and sodium inflow into the distal segment might thus be

Table II Case 2. Sodium transfers under different circumstances compared with the acute effect of mersalyl (μEq/min)

	Inflow	Reabsorbed
Mean 4 hrs mersalyl	1,468	757
1st day salt restriction	1,012	891
3 contr. hrs 4th day salt restr	830	803
Normal day with salt allowed.	1 138	1,014
During action chlorothiazide	1,049	727

The acute effect of mersalyl (fig 5)

In contradistinction to the foregoing experiments, this experiment was done after 3 days of salt restriction on which regimen sodium excretion had shown a step-wise decrease to a mean of 23 μEq/min. on the day before the experiment. This arrangement was chosen because the short-term experiment formed part of a more extended study to observe the effect on diabetes insipidus of sodium depletion by dietary restriction subsequently reinforced by repeated mersalyl injections. The first mersalyl injection, administered at moment when there was only slight sodium depletion, is presented here as a short term experiment (fig. 5)

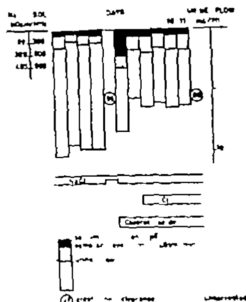


Fig. 6. Case 1 Long-term experiment with chlorothiazide Water and solute excretion before and during chlorothiazide After 5 control-days chlorothiazide was given for the next 6 days (0.5 g every 8 hours) Except for creatinine clearance, the data of day 5 has been omitted because of error in urine collection and salt administration. Ordinates for urine volume and solute excretion have been chosen in accordance with the normal relationship existing in plasma (1 ml contains 300 μOsm). Therefore the open parts of the columns indicate "free water" excretion.

Sodium reabsorption almost equals inflow during the control hours, i.e. sodium excretion is very low because of sodium restriction on the preceding days. Mersalyl is a slow-acting diuretic there is barely any effect after one hour Then an enormous increase of inflow occurs instead of the diurnal (afternoon) decrease which could be expected. Distal reabsorption does not follow this increase, nor does it show a decrease surpassing the blank experiment. There is an increase in urine-flow from 6 1 to 10.3 ml/min. The increased salt excretion seems to be solely the result of an important reduction in proximal sodium conservation. Creatinine clearances were determined twice during the short-term experiment. The value of 122 ml/min. during the action of mersalyl is perhaps significantly lower than the result during the control period in the morning (159 ml/min.) This morning value, however is unexpectedly high in a series of clearance values during the preceding and following days of the long-term experiment and we therefore hesitate to accept the difference as significant.

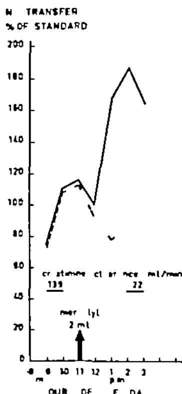


Fig. 5. Case 2. Acute effect of mersalyl on distal sodium inflow and reabsorption. Cf. legend fig. 1. Na inflow (——) N reabsorption (- -). Standard for transformation into percentage values is mean Na inflow from 8 a.m.—11 a.m. (830 μEq/min. = 100). The experiment was done after 3 days of salt restriction (Cf. fig. 8). At 11 a.m. 2 ml of mersalyl was injected intramuscularly.
Na-intake (8 a.m.—3 p.m.) nil
Na-excretion (8 a.m.—3 p.m.) 172 mEq

Completely blank experiments were done in both patients to evaluate influences of diurnal rhythm which might cause basic differences between the morning and afternoon hours (figs. 1 and 2 and table I). In these experiments we judged the sodium-inflow data of the last 3 or 4 hours (case 2 and 1 respectively) to be different from the initial hours and so used the mean of the respective first parts as a basis for calculation of percentage values. Both patients show a reduction of sodium inflow into the distal segment during the afternoon of about 80—85 % of the morning value. Sodium reabsorption in the distal segment follows a parallel trend in case 1. In case 2 there is a tendency to disproportionate decrease in distal sodium reabsorption, especially between 11 and 14 hours. No explanation for this slight difference can be offered. Creatinine clearance values are shown in the diagrams. In both patients there is no significant trend in the results of repeated clearance determinations.

Sodium inflow into the distal segment is the result of the combined actions of glomerulus and proximal tubules. Because the determination of a creatinine clearance is so inaccurate that errors of 15 ml/min may be present, no conclusions may be drawn from figs. 1 and 2 as to the exact localization of the mechanism of diurnal rhythm. It can only be said that these experiments suggest it to be the result of actions in the proximal part of the nephron.

Short-term experiments with chlorothiazide (figs. 3 and 4)

These experiments were done before the blank experiments described above. Regarded retrospectively our decision to introduce the variable (chlorothiazide) at 11 o'clock is disputable. The results, however, are clear enough to allow of interpretation.

Urine flows before and after chlorothiazide were 11.8 and 10.2 ml/min. in case 1 and 7.8 and 7.2 ml/min in case 2. Thus urine flow was hardly depressed by chlorothiazide during these experiments in which we compensated for sodium losses via the urine during the experiment.

Mean control sodium-inflow was calculated from the values of the first three hours. Depression of sodium-inflow in the afternoon (during the action of chlorothiazide) is still present and not significantly different from the effect of diurnal fluctuation as noted in the blank experiments. The effect of chlorothiazide on the distal reabsorption of sodium, however, is very obvious. Reabsorption is depressed to about 60 % of control-inflow or 72 and 69 % of the inflow prevailing during the afternoon hours, whereas in the morning hours 91 and 89 % of prevailing inflow were reabsorbed.

Increased sodium excretion (shown by the distance between broken and solid lines) as it results from the action of chlorothiazide in the dose given, seems to be solely the result of depression of sodium reabsorption in the distal tubular segment.

Table II Case 1. Sodium transfers under different circumstances compared with the acute effect of mersalyl (µEq min)

	Inflow	Reabsorbed
Mean 4 hrs mersalyl	*1,464*	*757*
1st day salt restriction	1,012	891
3 contr. hrs 4th day salt restr	830	823
Normal day with salt allowed	1 138	1,014
During action chlorothiazide	1 049	727

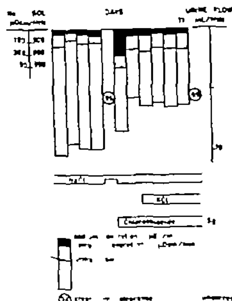


Fig. 6. Case 1 Long-term experiment with chlorothiazide Water and solute excretion before and during chlorothiazide. After 5 control-days chlorothiazide was given for the next 6 days (0.5 g every 8 hours) Except for creatinine clearance the data of day 3 have been omitted because of error in urine collection and salt administration. Ordinates for urine olume and solute excretion have been chosen in accordance with the normal relationship existing in plasma (1 ml contains 300 µOsm) Therefore the open parts of the columns indicate "free water excretion.

The acute effect of mersalyl (fig 5)

In contradistinction to the foregoing experiments, this experiment was done after 3 days of salt restriction on which regimen sodium excretion had shown a step-wise decrease to a mean of 23 µEq/min. on the day before the experiment. This arrangement was chosen because the short-term experiment formed part of a more extended study to observe the effect on diabetes insipidus of sodium depletion by dietary restriction subsequently reinforced by repeated mersalyl injections. The first mersalyl injection, administered at a moment when there was only slight sodium depletion, is presented here as a short-term experiment (fig 5)

Sodium reabsorption almost equals inflow during the control hours, i.e. sodium excretion is very low because of sodium restriction on the preceding days. Mersalyl is slow-acting diuretic there is barely any effect after one hour Then an enormous increase of inflow occurs instead of the diurnal (afternoon) decrease which could be expected. Distal reabsorption does not follow this increase, nor does it show a decrease surpassing the blank experiment. There is an increase in urine-flow from 6.1 to 10.3 ml/min. The increased salt excretion seems to be solely the result of an important reduction in proximal sodium conservation. Creatinine clearances were determined twice during the short term experiment. The value of 122 ml/min. during the ction of mersalyl is perhaps significantly lower than the result during the control period in the morning (139 ml/min.) This morning value, however is unexpectedly high in a series of clearance values during the preceding and following days of the long-term experiment and we therefore hesitate to accept the difference as significant.

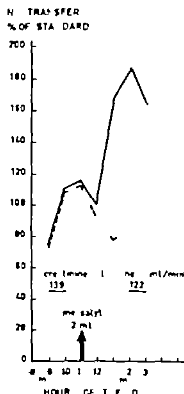


Fig. 5. Case 2. Acute effect of mersalyl on distal sodium inflow and reabsorption. Cf. legend fig. 1.
Na inflow (——) Na reabsorption (- - -)
Standard for transformation into percentage values is mean Na inflow from 8 a.m.—11 a.m. (830 µEq/min. = 100)
The experiment was done after 3 days of salt restriction (Cf. fig. 8). At 11 a.m. 2 ml of mersalyl was injected intramuscularly.
Na intake (8 a.m.—3 p.m.): nil.
Na-excretion (8 a.m.—3 p.m.): 172 mEq.

Completely blank experiments were done in both patients to evaluate influences of diurnal rhythm which might cause basic differences between the morning and afternoon hours (figs. 1 and 2 and table I). In these experiments we judged the sodium-inflow data of the last 3 or 4 hours (case 2 and 1 respectively) to be different from the initial hours and so used the mean of the respective first parts as a basis for calculation of percentage values. Both patients show a reduction of sodium inflow into the distal segment during the afternoon of about 80—85 % of the morning value. Sodium reabsorption in the distal segment follows a parallel trend in case 1. In case 2 there is a tendency to disproportionate decrease in distal sodium reabsorption, especially between 11 and 14 hours. No explanation for this slight difference can be offered. Creatinine clearance values are shown in the diagrams. In both patients there is no significant trend in the results of repeated clearance determinations.

Sodium inflow into the distal segment is the result of the combined actions of glomerulus and proximal tubules. Because the determination of a creatinine clearance is so inaccurate that errors of 15 ml/min. may be present, no conclusions may be drawn from figs. 1 and 2 as to the exact localization of the mechanism of diurnal rhythm. It can only be said that these experiments suggest it to be the result of actions in the proximal part of the nephron.

Short-term experiments with chlorothiazide (figs. 3 and 4)

These experiments were done before the blank experiments described above. Regarded retrospectively, our decision to introduce the variable (chlorothiazide) at 11 o'clock is disputable. The results, however, are clear enough to allow of interpretation.

Urine flows before and after chlorothiazide were 11.8 and 10.2 ml/min. in case 1 and 7.8 and 7.2 ml/min. in case 2. Thus urine flow was hardly depressed by chlorothiazide during these experiments in which we compensated for sodium losses via the urine during the experiment.

Mean control sodium inflow was calculated from the values of the first three hours. Depression of sodium inflow in the afternoon (during the action of chlorothiazide) is still present and not significantly different from the effect of diurnal fluctuation as noted in the blank experiments. The effect of chlorothiazide on the distal reabsorption of sodium, however, is very obvious. Reabsorption is depressed to about 60 % of control inflow or 72 and 69 % of the inflow prevailing during the afternoon hours, whereas in the morning hours 91 and 89 % of prevailing inflow were reabsorbed.

Increased sodium excretion (shown by the distance between broken and solid lines) as it results from the action of chlorothiazide in the dose given, seems to be solely the result of depression of sodium reabsorption in the distal tubular segment.

by the rearrangement of our data with calculation and presentation of $T_{Na,dist}$ and $Inf_{Na,dist}$ (fig 7) Mean sodium inflow into the distal segment during the 4 control days is used as basis for transfer into percentage values. A reduction of inflow and reabsorption of sodium to about 60 % results when the effect of chronic administration of chlorothiazide has allowed a new steady state to develop (days 8 to 11). This is accompanied by corresponding depression of urine-flow Day 6, in agreement with the acute experiments with chlorothiazide, shows that reduction of distal reabsorption is the primary event, while reduction of inflow turns out to be a later effect. It is fallacious to endow chlorothiazide with different properties in its immediate effects and during long-term administration, so it is tempting to look at the sodium losses of day 6 as the event initiating this reduction of inflow

The reported facts suggest that the nephron compensates for diminished sodium-conserving capacity in its distal segment by reducing the inflow of sodium into this segment. Because this compensation is sequel to previous sodium loss, it can only prevent further losses and cannot replenish them. Regulatory power of the proximal nephron in this respect seems to be a delicately-acting principle, since sodium losses on the 4 control and 4 stable experimental days averaged 58 and 61 μEq/min. respectively (dietary salt load unchanged) The experiment gives no clue as to the localization within the proximal nephron of this regulatory principle (glomerulus tubule).

Salt restriction and mersalyl

Earlier reports give conflicting results concerning the action of mercurial diuretics on urine flow in diabetes insipidus (2, 7 10) Because we supposed these discrepancies to be the result of the short action of mersalyl, which allows for sodium repletion during the remainder of an experimental day we kept our subject on a low sodium diet during the experiment. Three preceding days (fig. 8) show the result of simple salt-withdrawal. On days 4 and 5 a 2 ml intramuscular injection of mersalyl was given to increase salt-depletion. The effect of the first injection was studied in the experimental design of short-term experiments and discussed under that heading (fig 5) The results of the short-term experimental

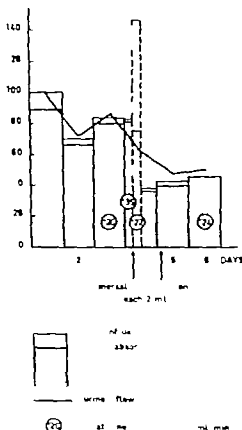


Fig 8 Case 2 Chronic effects of salt-restriction and mersalyl. Cf. fig 7 for arrangement of columns. Na inflow into distal tubules and its reabsorption there is expressed as percentages of the inflow value prevailing on day 1 (100 = 1,012 μEq min.). Urine-flow is given as percentage of the mean value over day 1 (100 = 7 4 ml/min.) The column of day 4 has been divided into 3 parts giving means for respectively the control hours before the short-term experiment, the experiment itself and the remainder of the 24 hour period (Cf. fig. 5 for short-term experiment)

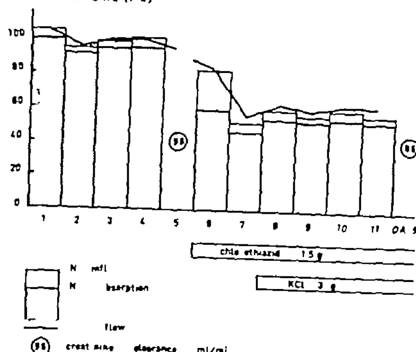


Fig. 7. Case I. Long-term experiment with chlorothiazide. Inflow of sodium into distal tubules and its reabsorption there are indicated respectively by superimposed open and hatched columns and expressed as percentage of a mean value for the inflow over the first 4 (control)-days (100 = 1.412 μEq/min.). Urine-flow is given as percentage of the mean value over the first 4 days (100 = 9.8 ml/min.). Cf. fig. 6.

It might be argued that in some way distal tubular reaction to this increase in inflow is inadequate in the face of rapidly increasing sodium losses and what is known to happen with comparable inflows in osmotic diuresis. This might indicate some toxic action of mersalyl on the distal segment. Comparison of the absolute amounts of sodium inflow and reabsorption during the action of mersalyl with the same quantities under diverse experimental conditions on other days (table II) strengthens this belief. Porush et al. (9) have recently discussed several possible theories to explain this phenomenon.

The experiment leads to the conclusion that mersalyl acts mainly or solely on the proximal part of the nephron. During the acute phase of its action it increases urine-flow in diabetes insipidus.

Long-term experiments with chlorothiazide

Fig. 6 gives a graphic account of water and solute excretion during the experimental days. Ordinate scale units indicate mean excretion of water and solutes in ml and μOsm per min. over 24hr periods. One scale unit represents 1 ml or 300 μOsm, the open parts of the urine-flow columns thus showing free water excretion. On day 5 a short supervised thirsting experiment was done. We regret that urine produced after this period was lost by negligence.

A short period of 2 or 3 days covers the disturbances in connection with the initiation of chlorothiazide therapy. Urine-volumes decrease significantly with chlorothiazide but not on the first day. A large loss of solute (mainly sodium) is recorded on that day. On the second and third experimental day sodium losses rapidly diminish and return exactly to the control value, to be from there on in balance with sodium intake. There is, however no recovery of lost sodium in the second part of the experimental period. From fig. 6 it may be concluded that chlorothiazide diminishes urine production in diabetes insipidus, but only after a lapse of time in which there is a considerable loss of sodium. The data suggest that chlorothiazide suppresses urine production *by way* of this sodium loss. Better insight into the mechanisms active during the episode under discussion is afforded

of the proximal tubule under all circumstances encountered in our experiments (3 12 13 14)

2) The diabetes insipidus in our subjects was complete, i.e. no A.D.H. was secreted.

3) There is (in the absence of A.D.H.) no basic water loss through the tubular lining of distal and collecting ducts.

4) Sodium and accompanying anion are the only solute reabsorbed in the distal part of the nephron, while NH and K (with anions) are the only solutes added to its contents. This addition amounts to the quantities found in the excreted urine.

5) There is a hypothetical, sharp boundary where the proximal type of isosmotic reabsorption is replaced by the distal reabsorption of solutes (Na) without any water movement in the absence of A.D.H.

The first presumption seems now to have been firmly established by the skillful micropuncture techniques of many investigators. Discussion of the second presumption serves little purpose because no decisive arguments can be offered to prove or disprove it. In our opinion the severity of the diabetes insipidus offered the same or a better guarantee of the absence of A.D.H. as found in many reported experiments in water-loaded subjects.

The possibility of basic water loss in the distal tubula segment in spite of the absence of A.D.H. has now been proven (1). The suggestion of Orloff and Walser (8) that osmolar concentration of the tubular contents influences this water transfer is very probable, but since their experiments do not preclude variations in the water inflow (V_i) their suggestion is not conclusively proven.

The presumption that sodium and its anion is virtually the only solute contributing to $T_{...}$ (i.e. reabsorbed) can be tested by looking more closely at solutes that might quantitatively be of some importance in this respect. The reabsorption of glucose is completed in the proximal tubule. Urea under circumstances of water diuresis is retained after filtration by the tubules in amounts of about 0.2 mOsm/min. If all this retention were accomplished in the distal part of the nephron it would not invalidate the results of our calculations by more than 100 μEq/min. of sodium transfer. Our results would then be higher than the true value to that extent. Other filtered solutes cannot compete in this respect with urea and glucose and therefore will not be discussed.

In the introductory paragraph the excretion of NH was estimated at maximal 50 μEq/min. Omission of this correction could have influenced our calculated sodium transfers to that amount in the opposite direction, i.e. depressing the calculated result. Since we used calculated osmolarity of the urine instead of direct measurement, this omission does not add to the inaccuracy.

The supposition of a sharp boundary between a proximal and distal type of solute reabsorption demands a strict anatomical definition. This is refuted by the widely divergent functions of the loop of Henle. Our way of treating experimental results divides the functions of this loop into two: isosmotic volume change and changes in osmolarity. Any over-all loss or gain in isosmotic volume will have been calculated as a loss or gain in sodium inflow into the distal segment. Any over-all solute balance deviating from isotonicity in reference to the volume balance will have been cal-

period are shown graphically in fig 8 in a separate column The experiment was stopped at the moment when mersalyl action had diminished to almost zero (sodium excretion during 4 hrs after mersalyl 171 mEq and during the remainder of the 24hr period 14 mEq) The results of the second mersalyl injection on day 5 were not studied separately it was given solely to promote further sodium loss. Fig 8 confirms in the results of days 1, 2 and 3 that simple sodium restriction is able to reduce urine flow in diabetes insipidus (broken line) as is widely known and shows that this reduction is achieved by a lowering of sodium inflow into the distal segment to 75 % of the control value in this experiment. This means that an important part of sodium conservation in response to dietary restriction is effected by the proximal part of the nephron (either glomerulus or tubule or both) A creatinine clearance was measured on day 3 and did not change significantly during the following experimental days (120 ml/min. on day 3 139 ml/min. on the morning of day 4 and 122 ml/min at the end of the experiment of that day On day 6 it was still 124 ml/min)

Day 4 (after the cessation of mersalyl effects) and days 5 and 6 demonstrate that repeated mersalyl injections by their aggravation of salt depletion reduce urine flow to 50 % of control value and sodium inflow into the distal segment by about the same amount. Sodium depletion is now severe enough to reduce excretion to virtually zero (conservation by distal tubule covering all of the inflow on day 6) It should be noted that on day 6 no mersalyl was given and the entire tubular system could be considered free of intoxication The data of this day thus show only the effects of severe sodium loss. There was no measurable reduction in creatinine clearance on that day (124 ml/min.)

It must be concluded that mersalyl is able to promote as a secondary effect a reduction of urine flow in a patient with diabetes insipidus under dietary salt restriction It acts by promoting salt depletion The study points to an important sodium-conserving mechanism in the glomerulus-proximal tubule complex.

These facts support the conclusions concerning the action of chronic chlorothiazide treatment The action of chlorothiazide is continuous and thus does not allow sodium repletion in spite of continuing sodium intake. The sodium loss acts however with chlorothiazide in the same way as in our mersalyl experiments by reducing inflow into the distal segment and thereby reducing urine flow

Further comments

In discussing the results we have refrained from indicating the proximal tubule as the site of regulation in proximal sodium conservation. Thus in spite of the lack of significant changes in creatinine clearances where measured The reader should note, however that in normal situations a glomerular filtrate of some 120 ml/min is reduced by the proximal tubule to about 10 ml/min. It is as yet impossible (and may well always remain an unsurmountable difficulty) to determine whether a further reduction to 7 or 5 ml/min is the result of a slight reduction of glomerular filtrate or of an increase of proximal tubular activity

Before discussing the pros and cons of our presentation of the experimental results, it is relevant to enumerate the presumptions on which it is based

1) Proximal tubular contents were isosmotic with plasma up to the last section

of the proximal tubule under all circumstances encountered in our experiments (3 12, 13 14)

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The effect of chlorothiazide is easier to obtain because of longer action and continuous administration, while mersalyl is short-acting and allows sodium repletion.

3) There is a powerful, quick-acting sodium-conserving mechanism in the proximal part of the nephron (glomerulus + prox. tubule)

4) Mersalyl acts mainly or solely as an *inhibitor of proximal sodium conservation*. Lack of compensating activity in the distal tubule may be due to a slight toxic effect of mersalyl on this part of the nephron.

5) In our experiments chlorothiazide acts only on the distal segment of the tubule by depressing sodium reabsorption.

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culated as a distal effect in sodium transfer.

The method of Heinemann et al. (4) for judging distal reabsorptive action from the excretion of "free water" is not entirely different from our method of handling experimental results. At any moment the flow of free water (C_{H_2O}) will be $\frac{V(P_{osm} - U_{osm})}{P_{osm}}$. Comparison with equation 5) shows that except for some minor corrections our calculations come near to translating free water excretion into sodium reabsorption in the distal segment. By doing this we enable ourselves to calculate sodium inflow into this segment, which is a valuable tool for judging the joint proximal actions of glomerulus and proximal tubule and at the same time offers a good basis for judging the distal reabsorptive effort.

To assess proximal function of the nephron in some of their experiments Heinemann et al. (4) use urine flow (V). Following our conclusions this may indeed be considered as a parameter because no volume change is supposed to take place in the distal segment and so V indicates not only urine flow but also the inflow of tubular urine into the distal segment ($V_1 = V = V$). Instead of adhering to this method we preferred to translate the experimental results into sodium transfers for the following reasons.

1) Although osmotically isotonic with plasma, proximal fluid when entering the distal segment need not be of identical solute composition. Under circumstances of very active proximal sodium reabsorption a higher urea and lower sodium concentration were conceivable. At least, an identical composition had to be proven. Our results seem to support this identity because fig. 8 shows that even under extreme salt-depletion with very active proximal sodium conservation urine flow is nearly parallel to changes in sodium inflow into the distal segment. To some extent this result is a confirmation and extension of the very recent work of Lassiter et al. (6) on the important flows of urea that rapidly equilibrate the urea content of the more distally situated segments with the prevailing interstitial urea concentration.

2) When sodium conservation by combined action of proximal and distal parts of the nephron is complete, as in our mersalyl experiment, the investigator who is accustomed to comparing urine flow with prevailing free water excretion will not be impressed by the fact that distal sodium reabsorption at that moment is at a maximum, unless he takes sodium excretion into account separately. There is a basic flow of sodium-free urine discernable under extreme sodium depletion which is not a fixed quantity but will vary with the urea production of the subject.

3) Without being any more inaccurate than the method of comparing urine flow and free water excretion, the calculated sodium transfers stress and illustrate to a much greater extent the basic events in which we are interested.

Summary

1) Under suitable conditions patients with diabetes insipidus can be used to study sodium conservation of proximal and distal parts of the nephron separately.

2) Reduction of urine flow in diabetes insipidus after mersalyl or with chlorothiazide are late effects. Their mechanism is not different from that of simple sodium deprivation and in fact must be the result of a sodium depletion.

The effect of chlorothiazide is easier to obtain because of longer action and continuous administration, while mersalyl is short-acting and allows sodium repletion.

3) There is a powerful, quick-acting sodium-conserving mechanism in the proximal part of the nephron (glomerulus + prox. tubule)

4) Mersalyl acts mainly or solely as an inhibitor of proximal sodium conservation. Lack of compensating activity in the distal tubule may be due to a slight toxic effect of mersalyl on this part of the nephron.

5) In our experiments chlorothiazide acts only on the distal segment of the tubule by depressing sodium reabsorption.

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In Vitro Studies on the Glucose Uptake and Fatty Acid Metabolism of Human Adipose Tissue in Diabetes Mellitus

A Preliminary Report

By

Lars A. Carlson and J. Östman

The increased fat mobilisation in diabetes mellitus has been better understood during the last decade through the discovery of the plasma free fatty acids (FFA) and the elucidation of their physiological importance (6, 7, 8). Close metabolic interrelations exist between carbohydrate and lipid metabolism in adipose tissue, which is the main source of the plasma FFA. A decrease of plasma FFA is thus obtained after glucose administration (6, 8) while fasting leads to increased FFA levels (6, 7, 8, 9, 12). It has correspondingly been shown *in vitro* that adipose tissue from fasting rats release more FFA than adipose tissue from fed animals (10, 17). The presence of glucose in the medium during *in vitro* incubation also depresses the release of FFA from adipose tissue (10).

The elevated plasma FFA levels in diabetes mellitus has been explained by an increased FFA release from the fat stores (11, 19). It has been supposed that a diminished uptake of glucose into adipose tissue or a decreased phosphorylation of glucose in adipose tissue (5, 16) might prevail in diabetes mellitus. This might lead to a decreased availability of α-glycerophosphate, the obligate glyceride-glycerol precursor (1, 14, 18, 21). There would consequently be a decreased esterification of FFA in adipose tissue. A higher release of FFA would then be a possible result of a decreased esterification.

The administration of insulin decreases the elevated FFA concentrations in diabetic subjects (2, 12). This effect has been ascribed to a diminished release of FFA from adipose tissue (3) secondary to a stimulation of the glucose uptake by insulin.

An increased FFA release has also been shown *in vitro* with adipose tissue from alloxan diabetic rats (20, 22). This elevated FFA release is partly inhibited when glucose is added to the incubation medium (4).

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Table II. Results from incubations of human subcutaneous adipose tissue in albumin Krebs-Henseleit buffer and Na-EDTA plasma for two hours. Mean values ± S. E. of mean are calculated from the average figure of two incubated tissues

	No.	Glucose uptake (mg/g)	Glycerol release (μM/ml/g)	FFA release (μEq/ml/g)	FFA uptake (μEq/ml/g)
Albumin medium					
Non diabetics.	6	1.04 ± 0.28	0.054 ± 0.012	0.059 ± 0.018	0.161 ± 0.036
Diabetics.	9	1.34 ± 0.18	0.109 ± 0.023	0.186 ± 0.037	0.208 ± 0.067
Significance of diff.		P > 0.05	P > 0.05	P < 0.05	P > 0.05
Plasma medium					
Non-diabetics	6	1.78 ± 0.28	0.086 ± 0.022	0.199 ± 0.047	0.208 ± 0.053
Diabetics	9	4.83 ± 1.17	0.185 ± 0.028	0.289 ± 0.061	0.329 ± 0.096
Significance of diff.		P < 0.05	P < 0.05	P > 0.05	P > 0.05

Table III. Individual differences in metabolic changes when adipose tissue pieces from one subject are incubated in Na-EDTA plasma (I) and albumin Krebs-Henseleit buffer (II) for non-diabetic and diabetic subjects. Mean difference ± S. E. of mean is calculated from the mean values obtained when two fat portions of one subject are incubated in (I) and two other fat pads are incubated in (II)

	No.	Glucose uptake (mg/g)	Glycerol release (μM/ml/g)	FFA release (μEq/ml/g)	FFA uptake (μEq/ml/g)
Non-diabetics					
Mean diff. ± S. E. of mean (I) — (II)	6	0.74 ± 0.077	0.036 ± 0.016	0.140 ± 0.055	0.047 ± 0.057
Significance of diff.		P < 0.05	P > 0.05	P > 0.05	P > 0.05
Diabetics					
Mean diff. ± S. E. of mean (I) — (II)	9	3.38 ± 1.13	0.083 ± 0.033	0.143 ± 0.061	0.120 ± 0.119
Significance of diff.		P < 0.05	P < 0.05	P < 0.05	P > 0.05

were obtained with the diabetic tissues. As glycerol is the final product of lipolysis, it probably serves as a good indicator of the lipolytic process. It should be pointed out that the direct comparison of glucose uptake as well as of net FFA release is difficult between the two groups. The reason is that the concentrations of glucose and FFA are different in the media of the two groups, these factors being known to influence the uptake of glucose as well as the release of FFA (1).

Table III presents the differences between the metabolic changes when the tissues of one subject are incubated in plasma and in albumin media respectively. The uptake of glucose is significantly higher during plasma incubation for both groups. The release of glycerol and FFA is significantly higher during plasma

Table I. Arterial levels of blood glucose, plasma glycerol and FFA at the time of the biopsy procedure. Mean values ± S. E. of mean

	No.	Glucose (mg/100 ml)	Glycerol (mM/l)	FFA (mEq/l)
Non-diabetics	6	85.5 ± 3.9	0.076 ± 0.006	0.55 ± 0.05
Diabetics	9	238.0 ± 29.1	0.092 ± 0.008	1.01 ± 0.10
Significance of diff.		P < 0.001	P > 0.05	P < 0.001

There being no reports on the relationship between carbohydrate and fatty acid metabolism of adipose tissue in human diabetes mellitus, the present studies were performed. Data from *in vitro* experiments are presented here on the uptake of glucose and the release of glycerol and FFA from human subcutaneous adipose tissue, for normal and diabetic subjects.

Material and methods

Six healthy men and nine male diabetics of various ages and different stages of clinical control were included in this study. None of the diabetics was seriously keto-acidotic and none was controlled only by dietary regime. The experiments were performed after fasting overnight and withdrawal of insulin or sulfonylurea for more than 24 hours in the six patients who were under treatment. Blood samples were withdrawn into heparinized syringes from an indwelling arterial catheter. During local lidocain anesthesia four specimens of subcutaneous fat each weighing about 300—500 mg were removed with minimal traumatization from the lateral aspect of the thigh. Two portions of fat were placed in flasks containing 6 ml of Na EDTA plasma simultaneously obtained from the subject. Two other fat pads were incubated in 6 ml of a Krebs-Henseleit bicarbonate buffer pH 7.4 containing 20 mg of human albumin and 1 mg of glucose per ml. In all media palmitic acid $1\text{-}^{14}C$ was added as the sodium salt (24). The incubation was performed at 37° C with air as gas phase. Changes of glucose and glycerol concentrations in the media were measured enzymatically (13, 15, 23). The FFA content in the media was determined according to Dole (6). The uptake of FFA was calculated as the multiple of percentage of initial radioactivity disappearing during the incubation and of initial FFA concentration. In six experiments the amount and radioactivity of the tissue lipids were also determined. After the fat tissues had been shaken in 15 ml of Dole extraction mixture (6) for 24 hours, the upper phase obtained by addition of water and heptane was taken for titration of FFA. By repeated washings with petroleum ether after alkalinization and acidification FFA and neutral lipids respectively were separated (24). The acid and the neutral fractions were assayed for radioactivity in a Packard Tricarb Liquid Scintillation Spectrometer. The entire procedure will be described in detail elsewhere (24).

Results

Blood glucose, plasma glycerol and FFA at the beginning of the study are given in table I. Blood glucose and plasma FFA were significantly higher in the diabetics.

The results from the incubation studies are presented in table II. During incubation in albumin medium the only difference observed between the two groups was a significantly higher net release of FFA from diabetic adipose tissue.

During incubation of the tissues in the subject's own plasma a higher uptake of glucose and a higher release of glycerol

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Zieve's Syndrome

Report of a Case

By

Justus Ström

In 1958 Zieve (2) described a syndrome characterized by transient hyperlipaemia, jaundice and haemolytic anaemia. The syndrome appeared in alcoholics and was associated with fatty liver and cirrhosis. The condition is probably not uncommon and is of interest from the aspects both of internal medicine and of surgery

Case report

A 45-year-old man. Regular abuse of alcohol for many years in form of half bottle of whisky a day. Has suffered from anxiety and feeling of inefficiency in his work. Hospitalized in 1960 for chronic alcoholism with polyneuritis. The cholesterol level was slightly elevated, 340 mg %, and the bromsulphalein retention 24.4 %.

Alcoholic abuse continued. His appetite deteriorated increasingly over a period of six months. 1 July 1961 he was very tired and had no appetite. Loss of 9 kg in weight in six months. No vomiting. In the week prior to admission he ran a pyrexia of 38—39° C. On July 14 friends noticed his jaundiced appearance. He was admitted to the Stockholm Hospital for Infectious Diseases on August 1 with suspected hepatitis. He had pronounced jaundice with 12 mg % bilirubin (for laboratory tests see fig. 1). Temperature 38.2. The liver was palpable three fingers below the costal margin. No ascites. The laboratory findings (Takata, prothrombin test, and gammaglobulin in serum) argued against cirrhosis. Epidemiological criteria for infectious or serum hepatitis were lacking, and normal thymol and GPT (GOT in first test 126, afterwards normal) was against a diagnosis of infectious hepatitis. Alkaline phosphatase on the other hand, was highly elevated in repeated tests (max. 28 units), so that a condition of stasis was suspected, and in particular cancer since no pain had been felt at any time.

Surgical intervention was considered, but it was decided to wait. The patient also had a urinary tract infection which was treated with sulphonamides and quickly cleared. His temperature fell below 38° but he was afterwards subfebrile for a fortnight or more. His condition gradually improved and the jaundice disappeared quite quickly. His sedimentation rate, initially 126 mm, fell slowly. *Cholecystography* on August 20 showed weak concentration of contrast medium in the gall bladder. No shadows of stones were seen. Moderately severe anaemia was found from the start (9.2 mg % Hb, 2.89 million red cells). Serum iron was normal (0.081 mg %), and reticulocytes likewise (3—11—6 ‰)

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incubation of the diabetic tissues. This difference is of borderline significance for the nondiabetic tissues.

In six experiments also the radioactivity of the tissue lipids was further studied A lower percentage of recovered radioactivity was found in the neutral lipid fraction of the diabetic tissues 54.2 % ± 7 7 (mean value ± SEM) compared to the normal tissues 75 8 % ± 7 6 when incubated in albumin medium For both groups a higher degree of esterification took place during plasma incubation where the corresponding figures were 66 0 % ± 8 1 and 84 4 % ± 5 1 respectively

Conclusion and summary

The methodology here outlined and to be described in detail elsewhere (24) seems to be satisfactory for measurements of metabolic changes in human adipose tissue, even if they are of less magnitude than in the rat. The technique with incubation of adipose tissue in albumin and plasma media might also be of value in differentiating between circulating plasma factors and tissue-bound factors

Although the studies here presented in a preliminary form are few in number some significant differences were found between the adipose tissue of non-diabetic and diabetic subjects. A higher net release of FFA (free fatty acids) and glycerol and a decreased esterification were found in diabetic tissues No relation was observed between the net release of FFA and the uptake of glucose per se This study thus strongly supports the theory that the elevated plasma FFA in diabetes is related to an increased release of FFA from adipose tissue No evidence was found that this disturbed lipid metabolism was related to a decreased glucose *uptake* in adipose tissue.

Acknowledgements

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The chief symptom is hyperlipaemia. In some cases it is so severe that the serum may be milky. Variations occur in respect of the fractions that are increased — neutral fat, fatty acids or phospholipids. An increase of cholesterol is admittedly usual, but may be absent. There is also the possibility of error in that, if cholesterol alone is determined, an increase may be thought to be due to obstructive jaundice.

Zieve regards the anaemia as being of a haemolytic nature caused by an abnormal lipid, probably lysolecithin. Kessel (1) too, considers this likely. He has also studied the erythrocyte survival, which he found to be diminished. The anaemia may be compensated by increased activity within the bone marrow which is usual in these cases. The relationship between anaemia and hyperlipaemia is also evident in that the anaemia quickly improves when the hyperlipaemia disappears. This happened also in the present case, in which no treatment for anaemia was given.

The third symptom, jaundice, is due principally to intrahepatic stasis with a varying degree of hepatocellular damage. This agrees well with the findings in our case. The alkaline phosphatase was high. The parenchymal injury on the other hand, was relatively slight, as shown by the figures of transaminase and by the bromsulphalein and galactose tolerance tests. The cirrhotic factor cannot be significant in this instance, as confirmed both by liver function tests and biopsy findings.

Zieve's syndrome is usually accompanied by pain, but was not in this case. As a rule the pain is epigastric, the site may vary but it is usually above the liver region. Characteristically it is episodic and cramplike lasting from a few minutes to hours. Between the attacks a dull pain often continues. In several cases the attacks have been so alarming that surgery has been performed. The explanation of the pain in hyperlipaemia is not known. Some have ascribed them to a fat embolism others have thought them to be associated with a sudden overloading of the liver and spleen with lipid substances, and refer to the severe abdominal symptoms in essential hyperlipaemia. This explanation is unacceptable, however since the pain persists for a long time after the hyperlipaemia has disappeared. The pain may be due to disturbances of lipid metabolism in the nerves or nerve cells similar to those found in diabetic neuropathies or in tabetic gastric crises. There is a toxic element, since all these patients with Zieve's syndrome are chronic alcoholics.

Kessel considers it unlikely that liver cirrhosis and fatty liver play an important role in the pathogenesis for there is no agreement between biopsy findings and symptoms. Patients with severe cirrhosis do not have this syndrome. The liver symptoms are simply evidence of chronic alcoholism. Kessel assumes that there must be simultaneously some damage of the pancreas, with insufficiency in production of a hormone which regulates the lipid threshold in the blood. He refers to certain animal experiments with cobaltous chloride in which the alpha cells are destroyed, leading to a temporary severe hypercholesterolaemia. The explanation in such case might be a simultaneous injury of the pancreas, causing hyperlipaemia, and of the liver giving rise primarily to intrahepatic cholestasis, which in turn prevents normal metabolism of the pathologically accumulated lipids.

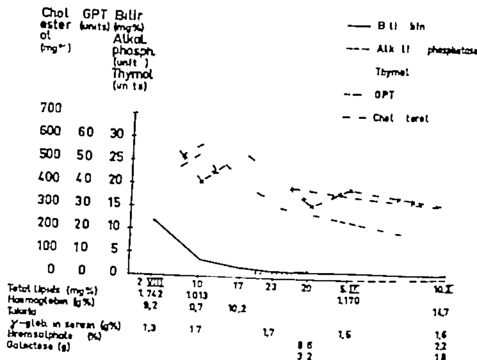


Fig 1 Laboratory analyses in case of Zieve's syndrome.

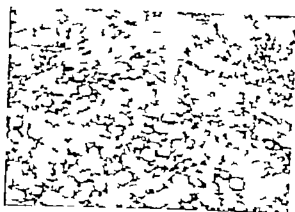


Fig 2. Liver biopsy

The cholesterol level in the blood was also high (686 mg against normal 250—300) Determination of total lipids on August 4 had shown 1 742 mg (normal 500—800) On August 10 the figure had fallen to 1 013 mg %. A closer analysis showed an increase of neutral fat (384 mg) total cholesterol (406 mg %) and fatty acids esterified with cholesterol (125 mg %) but low phosphatides (98 mg) After two months the cholesterol level was still rather high (fig 1)

Bromsulphalein and galactose tolerance tests of liver function one month after admission showed slightly elevated values, but returned to normal after a further month (fig 1)

Liver biopsy Aug 29 (fig 2) Abundant liver tissue, in which ordinary acinous structure is seen in places. Some acini, however appear to be deformed owing to an irregular fibrosis in the vascular zones. In some places the connective tissue has a tendency to divide up the acinous parenchyma into small balls. Moderately dense infiltration of lymphocytes is seen in the connective tissue. The parenchymal cells are rather irregular particularly because of very marked fatty accumulation without characteristic localization in the acini. No hepatomas were detected. The reticulo-endothelial cells are of ordinary appearance. PAD severe fatty liver and incipient cirrhosis.

The patient was given a hepatitic diet and vitamin B. His appetite gradually returned and his weight increased by over 2 kg in one month.

Discussion

The patient displayed the three cardinal symptoms of Zieve's syndrome — jaundice, hyperlipaemia and anaemia.

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Successful Cardiac Resuscitation in Myocardial Infarction

Report of a Case

By

Brostrup Knutsen, Magnus Tangen and Lorentz Gran

Sudden death in patients with cardiac infarction is often due to cardiac arrest which may occur in two major clinical forms ventricular standstill and ventricular fibrillation.

Wood (30) considers it likely that at least 1/3 of all patients with acute cardiac infarction die in this way as do about 10 % of the patients who live long enough to be hospitalized.

Often the autopsy is unable to provide any reasonable explanation for the sudden death. Out of 37 autopsies Miller (18) found that an adequate pathologico-anatomical explanation was available in 3 only who had myocardial rupture. In three other patients terminal ECG recordings showed ventricular fibrillation and eventual standstill.

Stroud and Fail (27) Hellerstein and Turell (12) and Enselberg (9) have all reported the terminal ECG-findings in patients with coronary heart disease who died suddenly showing either ventricular standstill or ventricular fibrillation.

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In the course of the past few years it has proved possible to resuscitate some of these patients. This is due to the fact that the disturbance in rhythm may be reversible while the myocardial damage in itself is not necessarily severe.

Case report

A 40-year-old man was admitted to the hospital on Sept. 9th 1961 In 1959 he had shown clinical and electrocardiographic signs of an acute anterior wall infarct. The following two to three months he had transitory moderate angina of effort.

The past three months he had had attacks of retrosternal discomfort, an almost burning sensation but not really a pain. The attacks were accompanied by weakness and sweating, and on one occasion he almost fainted. These attacks occurred at rest as well as on exertion and there was no known precipitating cause.

Clinical investigation, including the usual blood tests, urine tests, and X-ray of the heart, gave normal findings. The blood cholesterol was raised to 407 mg %. Blood pressure 120/80. ECG (standard limb leads and lead V4) showed atrio-ventricular conduction time 0.23 seconds, otherwise normal (fig. 1)

In the present case an acute infection in the urinary tract is likely to be a factor in provoking the syndrome. In Zieve's cases fever and cough were usually present.

In most instances the patients recover after four to six months. There is a marked tendency to relapse if the abuse continues. From the point of view of differential diagnosis it is of interest that in Zieve's cases cirrhosis had been diagnosed earlier 13 times obstructive jaundice and hepatitis 7 times each haemolytic anaemia 6 times, and pancreatitis 4 times.

Summary

A case of Zieve's syndrome is described in a chronic alcoholic with an acute attack of hyperlipaemia jaundice and haemolytic anaemia. Biopsy revealed fatty liver and incipient cirrhosis which however did not produce clinical symptoms nor was traceable in liver function tests The jaundice was caused primarily by intrahepatic stasis. A brief account is given of the symptomatology and of theories concerning the pathogenesis, and the differential diagnosis of the syndrome is discussed

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During this procedure cardiac arrest occurred once more, with respiratory failure and ventricular fibrillation. It now took less than minute to open the chest wall. Once again the heart was without visible activity. Ventricular fibrillation returned on further direct cardiac massage. Two further electrical shocks were given with the defibrillator and yet another 750 mg Pronestyl intravenously. After this there were one or two visible strong contractions at long intervals. After about two minutes massage the heart began to work regularly with good contractions (fig 6) and spontaneous respiration returned. The blood pressure was 115/70 and peripheral arterial pulse was good.

For the following two days the patient was treated under hypothermia. It was necessary to do tracheostomy because of obstruction of the air passages by secretions. In the course of the first 24 hours further disturbances in rhythm occurred, with transient auricular fibrillation (treated with Cedilanid) and 2—1 atrio-ventricular block with ventricular extrasystoles (treated with Isoprenalin).

His further progress was uneventful as far as the thoracotomy was concerned. However he developed right-sided pneumonia but this was not particularly severe (treated with antibiotics).

The patient remained unconscious for the first 30 hours. Consciousness then gradually returned, and after further two days he was fully conscious and psychically adequate.

Neurological investigation, including ECG, on Oct. 13th 1961 showed normal findings.

Neither the patient himself nor his nearest relatives have later noticed any personality changes. However on follow-up 4 months later there was still complete amnesia for the last 4 days before and for about the first three days after the cardiac arrest.

It was not possible to determine definitely whether the patient had had new infarct that caused the cardiac arrest, or whether the rhythm disturbances were the result of his previous infarct. The ECG changes on the following days were mainly those of pericarditis. The fever and raised ESR could have been due to his pneumonia. The SGO-transaminase rose from 12 to 167.

He returned to his previous work at the end of Feb. 1962, and since then has felt well.

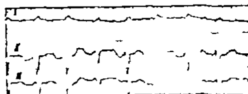


Fig. 5. ECG after thoracotomy and direct massage, 1st time.

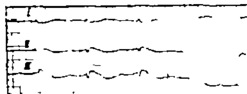


Fig. 6. ECG after direct massage, 2nd time.

Discussion

In this patient the cardiac arrest occurred under more or less optimal circumstances: he was under observation at the moment he lost consciousness, the rhythm disturbance could be recorded immediately electrocardiographically and qualified help and the necessary equipment were at hand in the course of few minutes. The majority of published cases of successful cardiac resuscitation have occurred under similar favourable conditions.

Animal experiments, as well as all clinical observations, have shown that effective circulation of oxygenated blood to the central nervous system must be established in the course of 3—5 or 3—6 minutes if irreversible cerebral damage is to be avoided. It is, therefore, essential that attempts at resuscitation should be begun promptly without waiting for electrocardiographical confirmation of which rhythm disturbance is present, and without using the decisive first minutes to prepare possible medical injections. Both of these must wait until other help is available.

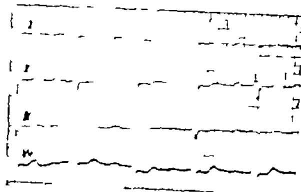


Fig 1 ECG before the exercise

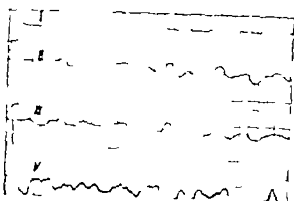


Fig 2 ECG 20—30 sec. fter the patient became unconscious.

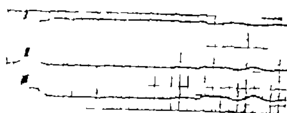


Fig 3 ECG 50—60 sec. after the patient became unconscious.

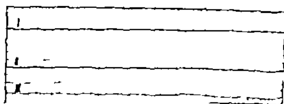


Fig 4 ECG during period of ventricular standstill.

On Sept. 12th the patient performed a two-step (Master) exercise test. He felt a little dizzy during the exercise, but was otherwise without complaint.

Just when the ECG was to be taken, immediately after the period of exercise was over the patient suddenly lost consciousness, respiration stopped after a few short gasps, his colour became livid, his pupils dilated. The blood pressure was not recordable and no peripheral arterial pulses were present.

Fig 1 shows the ECG taken before the exercise (unchanged from that on admission to hospital) Fig 2 is the first record taken 20—30 seconds after he became unconscious and fig 3 the ECG half a minute later

Closed chest cardiac massage was started between 2 and 3 minutes after he lost consciousness, and 0 75 mg adrenaline was injected intracardially He was given oxygen through a closed mask. This was replaced by tracheal tube and oxygen ventilation with a bag about 6—7 minutes after he lost consciousness when the anaesthetist arrived.

Closed chest massage and oxygen ventilation led to an undoubted improvement in his condition as his skin colour improved and the pupils contracted. The blood pressure was not measured at this time.

He continued to show mainly ventricular fibrillation (of the type shown in fig 2) with periods of ventricular standstill (fig 4). After the massage has been continued for about 10 minutes without the return of effective spontaneous cardiac rhythm, a surgeon carried out a thoracotomy and at the same time 750 mg Pronestyl was introduced slowly into a neck vein. The heart was found to be immobile but well contracted. After a few seconds of direct cardiac massage, visible ventricular fibrillation occurred. The direct massage was continued for 2 minutes without the return of effective spontaneous contractions. An electrical defibrillator was applied to the heart and a single electrical shock was given (220 V alternating current — 2 Amp — 0.5 sec.)

After defibrillation the heart was immobile, but after a few manual compressions spontaneous, regular strong contractions started (fig 5) The blood pressure was now 100/60, and the patient began to breathe. The chest wall was closed when his condition had remained stable for some minutes.

is the cause of the sudden cardiac arrest in at least 50 % of those with cardiac infarction.

Jude et al. (15) have used the closed chest technique systematically on 24 patients with cardiac infarction (21 had ventricular fibrillation and 3 ventricular standstill). Five patients were resuscitated to their pre-arrest central-nervous-system and cardiac status, and 3 patients left the hospital. Five patients had the massage initiated by ambulance attendants outside the hospital, and one of these survived.

Summary

A 40-year-old man, who had had an acute cardiac infarction two years previously developed acute cardiac arrest in connection with a two-step (Master) exercise test. The ECG showed ventricular fibrillation and ventricular standstill.

Closed chest cardiac massage was started between 2 and 3 minutes after he lost consciousness and oxygen ventilation after about 6—7 minutes.

Closed chest cardiac massage and oxygen ventilation led to an undoubted improvement in his condition. In addition he was given adrenaline intracardially and Pronestyl intravenously.

However he continued to show mainly ventricular fibrillation with periods of ventricular standstill. After the closed chest massage had been continued for about 10 minutes thoracotomy was carried out and the patient treated with electrical defibrillation and direct cardiac massage.

He remained unconscious for the next 30 hours. On follow-up 4 months later there was still complete amnesia for the last four days before and for about the first three days after the cardiac arrest. Otherwise he felt well, without any neurological or psychical disturbances, and was able to return to his previous job.

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The present patient should possibly have been ventilated by the mouth to-mouth method from the start until oxygen ventilation with a bag and tracheal tube could be established It is important to emphasize that the ventilation produced by closed chest cardiac massage is quite insufficient (8 25) and that concomitant artifical ventilation should always accompany the resuscitation procedure. The mouth to-mouth method has been found effective (17)

In the present patient it was obvious that the closed chest cardiac massage led to improvement in the patient's condition as judged from the skin colour and the contraction of the pupils. Observations with this method have shown that it is possible to establish effective blood pressure and peripheral arterial pulsation (13 16 23 25)

The method has already been widely used in cases of cardiac arrest under varying circumstances. Complications, in the form of rib damage have been reported (1 19) However Jude et al. (13) state that it is usually possible to avoid complications by applying the pressure correctly to the lower part of the sternum only They also state that the method if properly applied is harmless to a normally acting heart. This is of considerable importance when it is remembered that normal though weak heart action may be present in a collapsed patient.

Both in ventricular standstill and in ventricular fibrillation closed chest cardiac massage can provide physiological blood pressure levels and maintain viability of vital centres (6)

It is an open question to what extent closed chest cardiac massage alone can incite the heart to spontaneous activity in ventricular standstill. Quite often cardiotonic drugs have been used at the same time.

In ventricular fibrillation Keen (15) says that it is unlikely that the rhythm disturbance will be broken by closed chest cardiac massage. Julian (14) has reported that heart massage alone, in a very few cases, will be sufficient to stop ventricular fibrillation, and Walton (28) states that massage followed by intravenous Procain has stopped ventricular fibrillation. Electrical defibrillation seems now to be the most usual method in the treatment of ventricular fibrillation, in some cases combined with Pronestyl, Quinidine or Isoprenalin Nast et al (21) successfully applied external electrical defibrillation in a patient with cardiac infarction (3 electric countershocks 350 Volt of 60-cycle alternating current of 0 15 sec. duration each given at 10 sec. intervals) Julian (14) states that the external defibrillator must be able to deliver 780 Volts to the chest wall. As a rule electrical defibrillation will be effective only when the tone of the heart is good or has been improved by massage or cardiotonic drugs.

In the present patient, as in others (14) it was observed that electrical defibrillation was followed by cardiac standstill which quickly reacted to massage.

Zoll (31) has devised an external electrical pacemaker which has been recommended for routine use in myocardial infarction to treat sudden cardiac arrest (10 32) The instrument has since been combined with a device that monitors the cardiac electrical activity and, after a predetermined interval, actuates the pre-set external pacemaker when the electrical activity of the heart ceases. Straight et al. (26) suggest, however that the pacemaker is not started by ventricular fibrillation, which

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Aldosterone Treatment in Addison's Disease

By

Helge Worning and J. V. Roedbeck-Hansen

After it was realized that the most important mineralocorticoid of the adrenal cortex is aldosterone, it seemed natural to investigate the value of this agent in the substitution therapy of adrenocortical insufficiency. Several reports have appeared in recent years, but owing to the shortage of aldosterone the treatment periods have been very short. After the substance was synthesized, it became possible to extend the treatment over longer periods. The present paper reports on the experience of aldosterone as the mineralocorticoid in the substitution therapy of 4 women with confirmed Addison's disease. The treatment periods were up to 15 months.

Material and methods

Table I gives the patients' data and their previous substitution therapy.

In all cases the diagnosis was based upon the presence of fatigue, weight loss, typical pigmentation, low blood pressure, a low serum sodium, and the lack of any increase in the urinary excretion of 17 ketosteroids and 17-ketogenic steroids following parenteral administration of 25—50 units ACTH.

Submitted for publication February 14, 1963.

The investigations fall into 3 parts.

In *Part I*, comprising cases A, B and C, the effect of d-aldosterone acetate administered orally in the form of tablets was compared with that of desoxycorticosterone trimethyl acetate (Percorten®) administered i. m. All the patients were in-patients, but ambulatory and on an ordinary diet.

During the 1st period they received their usual substitution therapy.

During the 2nd, 3rd, and 4th periods they received 3, 4.5, and 6 mg aldosterone respectively in 3 divided doses daily instead of Percorten. The dose of cortisone was kept unchanged. These 3 patients were then followed as out-patients, still receiving aldosterone as mineralocorticoid. Case A was readmitted after 7 months' treatment as described under Part II.

Part II comprises cases A and D both of whom were in-patients, but ambulatory. In these cases the intake of sodium chloride was known.

During the 1st treatment period case A received aldosterone by mouth, while case D received her usual substitution therapy. During the next period both received d-aldosterone subcutaneously (Aldocorten®) 1 and 1/2 mg respectively in daily doses. During the 3rd period they received d-aldosterone sublingually in 3 daily doses.

During the 1st and 2nd part of the investigation the urinary output and urinary excretion of sodium, potassium, and cloride were

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				Percorten® i.m. (mg)	Decortone® sublingually (mg daily)
A	44	1.5	25	25/2 weeks	—
B	66	11	25	50/3 weeks	—
C	62	12	18.5	50/3 weeks	—
D	53	10	25	25/10 days	1

determined daily Weight, blood pressure, plasma electrolyte concentration, serum creatinine, red cell volume, total serum protein, fasting blood sugar eosinophil count in peripheral blood, and the 24-hour urinary output of 17 ketosteroids and 17 ketogenic steroids were determined twice weekly

The extrarenal loss of sodium chloride was estimated to be 25 mEq/24 hours in Case A and 15 mEq/24 hours in case D The difference is due to A's greater body surface and amount of sweat.

Part III comprising cases A, B, and C, represents the out patient follow-up on permanent aldosterone medication in the form of d aldosterone acetate, either by mouth or sublingually In this part of the study weight, blood pressure, and plasma electrolyte concentration were determined at irregular intervals.

Biochemical methods Sodium and potassium by flame photometry Chlorides by complexometric titration by the method of Chales and Chales. Serum creatinine by the alkaline-picrate method, blood sugar by the Hagedorn-Norman Jensen method. Serum protein by the biuret method, 17-ketosteroids by a spectrophotometric micromethod, and 17 ketogenic steroids as 17 ketosteroids after reduction of the latter by sodium borohydride and oxidation of 17-ketogenic steroids by bismuthate.

Results

Part I

Table II shows the daily urinary excretions of sodium, chloride, and potassium during the 4 treatment periods,

Table II Urinary output of electrolytes on various mineralocorticoids

Pat.	Period of treatment	Days	Urinary output (mEq/24 hours)					
			Sodium		Chloride		Potassium	
			Mean	S. D	Mean	S. D.	Mean	S. D.
A	1	19	87	26	91	25	28	7
	2	5	85	30	89	32	37	10
	3	9	70	11	75	14	35	5
	4	8	54	16	62	17	26	4
B	1	13	82	21	94	24	35	3
	2	10	72	20	91	21	34	5
	3	10	91	22	121	44	36	6
	4	9	89	32	101	30	33	6
C	1	11	88	31	100	25	53	7
	2	10	96	30	106	21	56	6
	3	10	103	28	126	31	57	7
	4	9	119	19	128	17	57	7

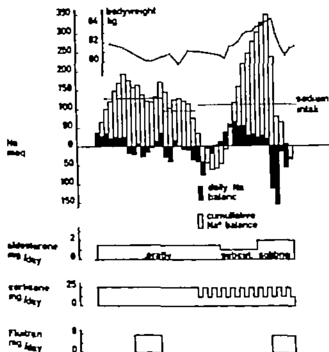


Fig. 1 Case A. Body weight and sodium balance, daily and cumulative, during aldosterone therapy

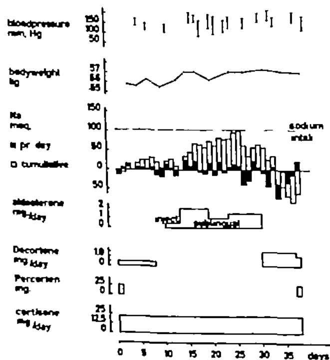


Fig. 2. Case D. Blood pressure, body weight, and sodium balance, daily and cumulative, during treatment with various mineralocorticoids.

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	2	10	72	20	91	21	34	5
	3	10	91	22	121	44	36	6
	4	9	89	32	101	30	33	6
C	1	11	88	31	100	25	53	7
	2	10	96	30	106	21	56	6
	3	10	103	28	126	31	57	7
	4	9	119	19	128	17	57	7

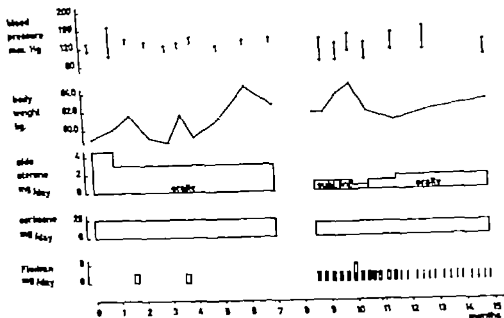


Fig. 3. Case A. Blood pressure and body weight during long-term aldosterone therapy

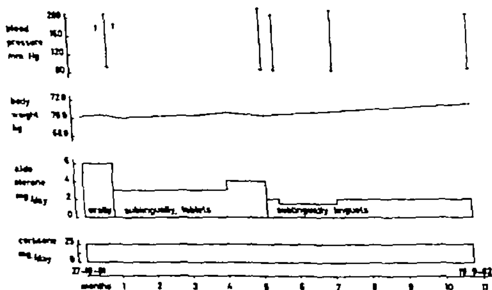


Fig. 4. Case B. Blood pressure and body weight during long-term aldosterone therapy

calculated as mean values and standard deviation Case A shows a sodium and chloride excretion decreasing as the dose of aldosterone was increased. This fall was significant at the 5 per cent level on administration of a daily dose of 4.5 and 6 mg aldosterone respectively No changes were found in the potassium excretion. Case B shows no alterations in the electrolyte excretion whereas case C shows a significantly increased sodium and chloride excretion ($p < 0\ 05$) on 6 mg aldosterone. No changes in potassium excretion In case A the blood pressure tended to rise slightly on the maximum dose of aldosterone but otherwise the blood pressure, body weight urinary output total serum protein red cell volume, serum creatinine, fasting blood sugar eosinophil leukocyte count and urinary excretion of hormones remained unchanged throughout the period of the study

Part II

Figs. 1 and 2 show the results of balance studies in cases A and D In case A 1 5 mg aldosterone by mouth appears to keep up the sodium balance if anything there was some retention of sodium on a daily intake of 128 mEq sodium Owing to weight gain and oedema the patient was treated with a thiazide derivative, but ineffectively However a reduction of the daily sodium intake to 94 mEq resulted in a negative sodium balance which did not definitely alter when sodium intake had been increased to 111 mEq daily Alteration of the aldosterone therapy to 1 mg subcutaneously daily immediately resulted in a considerable retention of sodium which remained unchanged on 2 mg aldosterone sublingually The sodium retention (a total of 345 mEq) caused a marked weight gain and perceptible oedema. This again necessitated the administration of a thiazide diuretic. On this medication the retained amount of sodium was excreted, and at the same time the weight decreased. The level of chloride paralleled that of sodium. The blood pressure was constant, as were the potassium excretion, plasma electrolyte level, urinary excretion of hormones, and other recorded values.

In case D the usual substitution therapy had maintained the sodium balance. The change to aldosterone, first subcutaneously and then sublingually resulted in some sodium retention and associated weight gain and a considerable increase in blood pressure, entailing subjective discomfort in the form of headache and dizziness which made it necessary to withdraw aldosterone. An attempt to treat her with desoxycorticosterone exclusively by sublingual route, entailed a considerable sodium excretion, and a balance was not achieved until the treatment had been supplemented by Percorten i m. In this case too, the chloride level was parallel to that of sodium. No definite alterations were found in plasma electrolytes, daily output of urine, red cell volume, total serum protein, serum creatinine, fasting blood sugar eosinophil count in the blood, or urinary excretion of hormones.

Part III

The results of the long term treatment are shown in figs. 3 4 and 5 In case A the body weight increased steadily on oral administration of 3 mg aldosterone daily Sublingual treatment could be carried through only for a short time because of the bitter taste of the tablets. For a time subcutaneous injections were tried Weight gain during this treatment

Fig. 3. Case A. Blood pressure and body weight during long-term aldosterone therapy

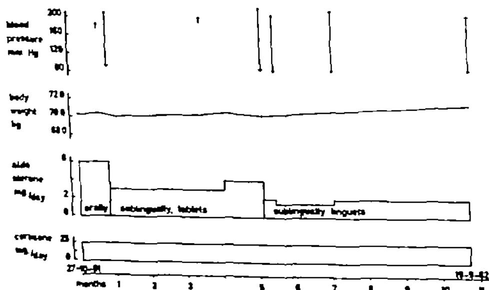


Fig. 4 Case B. Blood pressure and body weight during long-term aldosterone therapy

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blood pressure mm Hg
body weight kg
aldo-sterone mg/day
orally
subl. lin.
cortisone mg/day
months
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

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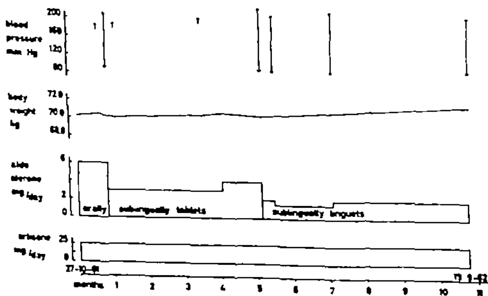


Fig. 4. Case B. Blood pressure and body weight during long-term aldosterone therapy

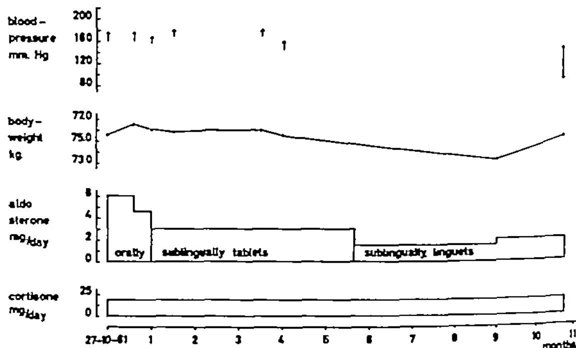


Fig 5 Case C. Blood pressure and body weight during long term aldosterone therapy

made it necessary to resume oral medication. In cases B and C the treatment was by the sublingual route except during the initial period.

The doses of aldosterone were adapted to the general condition, especially the sensation of fatigue. The dosage required to reduce the fatigue as far as possible entailed in case A a tendency to oedema, so this patient had to be treated at intervals with diuretics. In the other two patients the body weight remained constant, and in all cases the blood pressure and plasma electrolyte concentration remained within the previous range. These findings indicate that the treatment has been able to keep the patients in sodium balance.

Effect on pigmentation and general condition

In all cases the pigmentation faded during the first months of the treatment, but without the colour returning to normal.

The effect upon the general condition has been striking, especially in case C whose fatigue has yielded after she was switched over from desoxycorticosterone to aldosterone. Cases A and B are also feeling better on aldosterone, although the difference is not so marked but none of the patients wants to return to their previous treatment.

Discussion

The dose of aldosterone required to ensure a complete substitution in adrenocortical insufficiency has not been finally fixed. According to Ulick et al. (9) the normal aldosterone production on an ordinary diet is between 150 and 300 µg daily but depending largely on the intake of sodium. The fact that Kekwick

and Pawan (3) succeeded in achieving a sodium balance in an Addisonian on a daily oral dose of 100 μg aldosterone in solution can mean only one thing, viz. that the endogenous production of aldosterone has not been completely abolished as oral medication is considerably less effective than parenteral administration owing to the passage of aldosterone through the liver and its partial inactivation in this organ (2, 4). Prunty et al. (7) as well as Mach et al. (5) found the substitution dosage by intramuscular injection to be 150—300 μg in Addison's disease, while in one case of adrenalectomy Ledingham et al. (4) found that 1 mg d l-aldosterone i. m. was sufficient.

Only a few therapeutic experiments with oral aldosterone acetate have been reported. Ledingham et al. (4) obtained sodium retention in 2 adrenalectomized women on 5 and 10 mg respectively in the 24 hours, whereas Nelson and Cooper (6) report that 900 μg aldosterone acetate by mouth was unable to set up sodium balance in an Addisonian. In this case, however, a balance was achieved on 600 μg aldosterone acetate sublingually.

The present results correspond well with these previous findings. By subcutaneous injection we obtained a considerable retention of sodium on 1 mg (fig. 1) and a sodium balance on 1/2 mg (fig. 2) daily indicating that this route is as effective as the intramuscular.

With oral administration the requirement is between 1.5 and 6 mg daily but on the basis of the results reported so far it is impossible to fix an actual minimum dosage. It is difficult to decide whether the increasing sodium and chloride excretion in case O (table II) on increasing aldosterone dosage reflects an insufficient mineralocorticoid effect, as the patient was on an ordinary diet, and it is therefore impossible to exclude a somewhat greater intake of salt on these days.

Sublingual application was effective in all cases in doses of 1—2 mg daily. In this respect too the experimental design does not permit the fixing of an actual minimum dosage, but as in the case of oral administration there seems to be a question of considerable individual variations.

The results do not indicate that the effect of aldosterone differs from that of desoxycorticosterone in respect to fluid distribution, carbohydrate turnover, eosinophil count in the peripheral blood, renal function, or urinary excretion of hormones. Aldosterone is able to normalize the blood pressure in adrenocortical insufficiency (3), but as a general rule it does not exert a hypertensive effect. Thus, Rosemberg et al. (8) found no effect upon the blood pressure of intramuscular injections of 10 mg d-aldosterone daily for 2 weeks into normal subjects. On the other hand August et al. (1) in two normal subjects, treated with d,l-aldosterone 3 mg i. m. daily observed an increase in the diastolic blood pressure between 10 and 20 mm Hg in the period with maximal sodium retention, and the therapeutic results in our case D show that a rise of blood pressure may render continued aldosterone therapy difficult.

Both Mach et al. (5) and Kekwick and Pawan (3) have reported an improvement in the general condition of Addisonian patients on aldosterone therapy. In the present study all 3 patients on long-term therapy exhibited definite improvement, manifesting itself particularly in a decreased fatigue after the medication had been altered from Percorten to aldosterone.

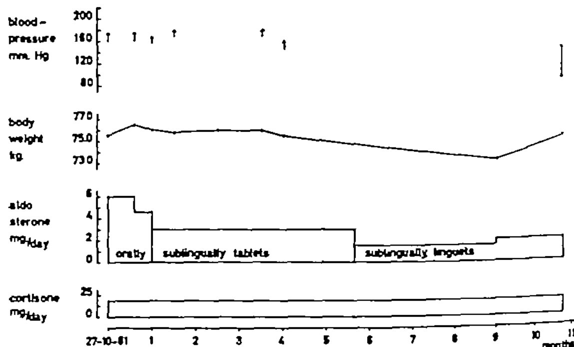


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Depigmentation during aldosterone therapy has previously been observed by Mach et al. (5) and Kekwick and Pawan (3)

Summary

The metabolic effect of d aldosterone administered subcutaneously (Aldocorten®) sublingually or orally as d-aldosterone acetate, was investigated in 4 women with Addison's disease of 18 months to 12 years duration on continuous cortisone medication A sodium balance was achieved in all the cases. This was obtained by aldosterone 1/2—1 mg when administered subcutaneously 1—2 mg when administered sublingually and 1/2—6 mg when administered orally

In one case the treatment resulted in such a marked rise of blood pressure that aldosterone had to be discontinued

The remaining 3 patients were followed on an out patient basis on continued aldosterone therapy for 11 11 and 15 months During these periods their general condition has been better than previously when they were treated with desoxycorticosterone trimethyl acetate (Percorten®) and the pigmentation has faded somewhat.

In one of the cases a tendency to oedema has necessitated a frequent administration of a diuretic, but apart from this her weight, blood pressure, and plasma electrolyte concentration have been satisfactory

It may be concluded that aldosterone is an important adjunct in the substitution therapy of Addison's disease.

Acknowledgement

The authors wish to thank Dr J Lorenzen, Ciba Ltd Copenhagen, for supplying the aldosterone used in this study

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Aetiologic Agents in Respiratory Illness

Occurrence of Bacteria and of Serologic Reactions Against Viruses and Bacteria in Acute Respiratory Illness

By

G. Tunevall, M. Ohlson, A. Svedmyr, G. von Zeipel, Å. Frisk, P. Hedlund, B. Lambrager and H. Jerkelius

Adequate handling of acute respiratory illness and especially the evaluation of methods for their prevention and treatment should be based on a good understanding of aetiologic factors involved. Studies on such factors have certainly been numerous but have usually covered one sector only of the entity of respiratory disease or one group only of pathogenic agents.

Combined virologic and bacteriologic investigations of cases of acute respiratory illnesses have been made (e.g. 1, 4, 7, 10, 13, 15, 19, 20) and the aetiological significance of a number of viruses has thereby been elucidated by virus isolation as well as by serologic methods. When it comes to bacteria their incidences have generally been close to those found in so-called normal materials (7, 10, 13, 15) and serologic tests have not been available or at least not used in order to throw light upon the importance of bacterial infection.

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Such tests were used by Tunevall in studies on otorhinologic (26) and acute respiratory (28) illness in children. In these investigations, however, virus infections were not studied. The same set of serologic tests was employed by Philipson as a complement to virus studies on non-diphtheritic croup (17, 18) and by Dinter et al. in investigations of acute respiratory illness in conscripts (6). Sterner and Tunevall have recently reported a sero-bacteriologic and -virologic study on acute respiratory illness in children (23).

In the present investigation patients hospitalized for acute respiratory illnesses have been examined with repeated bacteriologic cultures and by serologic tests for a number of viral and bacterial agents. Frequent sampling for cultures and a fairly large number of serologic tests were made in order to give a better chance of demonstrating the presence of potentially pathogenic agents. Cultures for fungi

were also made, and all sera were tested for agglutinating and complement fixing antibodies against Candida albicans but few isolations and no significant titre changes were recorded. The mycologic results are left out in the following as being uninformative.

Material

Two groups were included in the investigation. Group I contained patients picked out at random among all cases with symptoms of acute respiratory illness admitted to two of the four reception wards of the hospital from March 1957 through March 1958. The latest part of this period coincided with the 1957—58 epidemic of influenza A_2 in Stockholm. Of 130 patients chosen for this study only 78 were hospitalized long enough for the scheduled samples to be taken.

Group II, 124 patients, 82 of whom could be adequately followed, was collected during the short period of April, May and early June 1958, coinciding with the later part of the influenza A_2 epidemic.

The degree to which the patients examined were representative of the entire material of acute respiratory illness in the hospital during the same period is illustrated in tables I and II. The age distributions of the investigated patients, the patients who had to be omitted from the chosen groups, and all other patients admitted for acute respiratory illnesses during the same period are given in table I. The youngest age group is underrepresented in the groups examined. On the other hand, the number of aged persons was comparatively high among the investigated patients of group II, possibly because such persons more commonly stayed in hospital long enough to permit the scheduled samples to be taken.

As shown in table II, a comparatively large proportion of patients with uncomplicated respiratory illness escaped adequate follow-up. Consequently the incidence of pneumonia was higher in the groups investigated than among other patients.

Methods

Attempts at virus isolation from stool specimens (taken as early as possible after admission) were made only in cases displaying serologic reactions against adenovirus. The technique employed is described elsewhere (22). Bacterial cultures were made according to customary technique on the day of admission and repeated on the second, fifth, and tenth days as a minimum schedule. On each occasion nasal, naso-pharyngeal, and throat swabs were cultured, and sputum samples when available, occasionally also secretions from paranasal sinuses and middle ears. Findings from the air passages are reported only when made in direct plate cultures, whereas results of enriching cultures are left out.

Blood was drawn for serologic tests on the day of admission and on approximately the fourteenth day. Enough blood was taken for the following tests to be done:

Infection with.	*Test.*
Influenza A and B viruses	Complement fixation (soluble antigen)
Adenovirus	Complement fixation (soluble antigen)
Psittacosis virus	Complement fixation
Eaton's agent (primary atypical pneumonia)	Cold agglutination
Infectious mononucleosis agent(s)	Paul-Bunnell's and Davidsohn's tests
Pneumococci	Antipneumolysin (APn) (27)
β-streptococci	Antistreptolysin (AS) (11, 12)
Staphylococcus aureus	Antistaphylolysin (ASta) (16)
Haemophilus influenzae	Complement fixation (AHI) (25)
Escherichia coli	Anticolilysin (ACol) (29)
Candida albicans	Agglutination and complement fixation (2, 8)

Titres were considered significantly changed if, for antilysin and agglutination tests, increased more than two-fold, for other tests four-fold. In addition, cold agglutinin titres above 1/16 and Paul/Bunnell titres (after absorption) above 1/160 were considered indicative of recent infection. Except for cold agglutinins and Paul-Bunnell titres the two samples from every patient were always tested together.

Table I. Age distribution of the examined groups I and II of patients excluded from these groups, and of all other patients with acute respiratory disease admitted during the observation time

	Age (yrs)				
	< 15	15—39	40—59	≥ 60	Total
Group I. Examined	8	28	24	18	78
Excluded	8	18	15	11	52
Group II Examined	5	18	19	42	82
Excluded	8	14	10	10	42
Others	149	221	203	341	914

Table II. Incidence of pneumonia and purulent complications (e. g. sinusitis, otitis, pulmonary abscess) in the examined groups I and II patients excluded therefrom, and on all other patients with acute respiratory disease admitted during the observation time

	Clinical diagnosis		
	No pneumonia	Pneumonia	Purulent complications
Group I. Examined	21	57	8
Excluded	21	29	2
Group II. Examined	28	54	7
Excluded	19	19	4
Others	332	468	114

The significance of decreasing titres

In the bacteriologic study an evaluation of titre decreases of the same magnitude as set out above for increases has been attempted. Titre decreases were rather common, probably because the time elapsed from the onset of disease to admission was in some cases rather long.

A titre decrease to less than half the initial value in about fortnight would indicate the existence of a fairly steep descending branch of the titre curve. In order to find out if and when such descending branch can at all be observed in the present material, the APn values have been arranged in fig. 1 as functions of the time elapsed from onset of disease to sampling. The APn reaction was the only one displaying variation big enough for such an analysis. Titres recorded in patients harbouring no or few pneumococci are given separately from those found in patients with abundant growth. Among the latter titres of pneumonic patients are reported separately from those of non-pneumonic ones.

The titres of patients with no or few pneumococci were evenly distributed during the whole observation time. Non-pneumonic patients with pneumococci had titre averages which for the period between the sixth and the fifteenth days exceeded those of the former group (t-value in Student's test 2.79, $P < 0.01$). Pneumonic carriers of pneumococci presented both higher and more prolonged elevation, significant for the period between the sixth and the twenty-fifth days ($t = 8.71$ $P < 0.001$). In this group a descending part of the mean titre curve could be established, as the mean titre after the twenty-fifth day was significantly below that of the period from the eleventh to the twenty-fifth day ($t = 3.40$, $P < 0.01$).

In table III the numbers of increasing titres versus the decreasing ones are given for patients with different periods of illness preceding the first sampling. As bacteria resistant to penicillin were, as compared with sensitive ones, generally found later in the

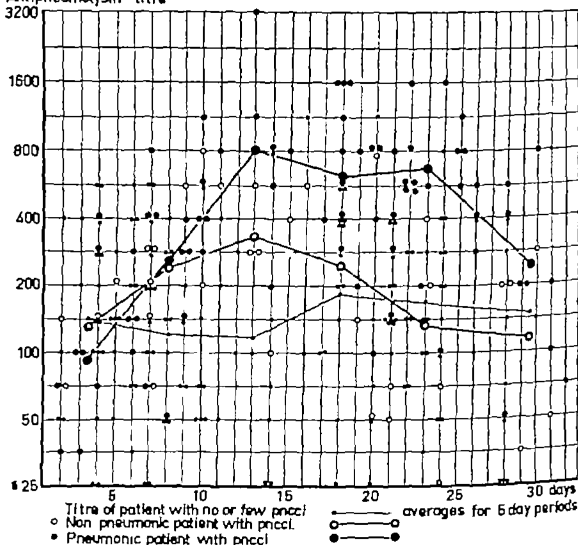


Fig 1 Antipneumolysin titres recorded after different periods of illness counted from the day of onset of the disease. Patients yielding no or only few pneumococci in cultures from nose, throat, nasopharynx, or expectorate are reported separately from pneumococcal carriers. Among the latter nonpneumonic patients are separated from pneumonic ones. The geometric means of the titres are calculated for each group and for each 5-day period and incorporated in mean curves for the three groups.

course of disease (cf. table VIII) perhaps often as a result of nosocomial infection, reactions against sensitive bacteria are reported separately from those against resistant organisms. Against sensitive bacteria titre increases were most common in patients admitted and examined early decreases more common among those admitted later in the course of disease. For reactions against resistant bacteria increases became more frequent in later stages and decreases did not occur at all. Thus, the time distribution of the reactions agreed with that of the occurrence of the provoking bacteria.

Consequently a drop in titre against bacterial antigens will be regarded below as similar in significance to an increase.

Accordingly the numbers of "reactors" given below represent

a) patients with significant titre rises,

b) patients with cold agglutinin titres above 1/16 and Paul-Bunnell titres above 1/160, and

c) patients with significant decreases in antibody titres against bacterial antigens.

Table III. Incidence of increasing versus decreasing antibody titres, and of reactions not significantly changed in spite of the corresponding bacteria being present in cultures, in patients having been ill for periods of different length prior to first sampling. Reactions against penicillin-sensitive (Gram-positive) bacteria are reported separately from those directed against penicillin-resistant ones (Gram-negatives and resistant St. aureus strains)

Period of illness before first sampling (days)	Penicillin-sensitive			Penicillin-resistant		
	Increasing	Unchanged	Decreasing	Increasing	Unchanged	Decreasing
2–4 (37 cases)	24	7	0	6	9	0
5–7 (50 cases)	19	11	6	6	7	0
8–10 (32 cases)	13	12	9	13	6	0
>10 (41 cases)	15	8	10	14	5	0

Table IV. The incidences of serologic reactions against virus as well as non specific reactions among non-pneumonic versus pneumonic patients in groups I and II. The numbers of cases with simultaneous reactions against bacteria are given in brackets

Serologic reaction against	Group I			Group II			Group I + II		
	Non-pneu-monic	Pneu-monic	Total	Non-pneu-monic	Pneu-monic	Total	Non-pneu-monic	Pneu-monic	Total
Influenza A & B	5 (3)	12 (9)	17 (12)	4 (3)	9 (9)	13 (12)	9 (6)	21 (12)	30 (24)
Adenovirus	—	1 (1)	1 (1)	1 (1)	1 (1)	2 (2)	1 (1)	2 (2)	3 (3)
Psittacosis	—	—	—	1 (1)	2 (2)	3 (3)	1 (1)	2 (2)	3 (3)
Cold agglutin.	1 (1)	3 (1)	4 (2)	1 (1)	6 (4)	7 (3)	2 (2)	9 (5)	11 (7)
Paul-Bunnell	—	—	—	3 (0)	—	3 (0)	3 (0)	—	3 (0)
Total	6 (4)	16 (11)	22 (15)	10 (6)	18 (16)	28 (22)	16 (10)	34 (27)	50 (37)
No. of cases	21	57	78	28	54	82	49	111	160

Results

Virological

For simplicity we have under this heading included the customary serologic tests for primary atypical pneumonia and mononucleosis, although Eaton's agent, the probable cause of cold-agglutinin-positive primary atypical pneumonia, has recently been found to be a mycoplasma (3, 5, 14) and little if anything is known about the aetiology of infectious mononucleosis.

A summary of the incidences of serologic reactions indicative of fresh infection is given in table IV. The numbers of cases in which reactions were found also against bacteria are given in brackets. Recent infections with viruses or mycoplasma were evident in 50 of the 160 cases (31 %). Cold agglutinin reactions were frequent among pneumonic patients whereas Paul-Bunnell reactions occurred only in non-pneumonic ones.

Bacteriological

An enumeration of the serologic reactions against bacteria is given in table V

Table I. The total incidences of serologic reactions against bacteria. The numbers of descending titres included in the totals are given in brackets

Serologic reaction against	Group I			Group II			Group I + II		
	Non pneumonic	Pneumonic	Total	Non-pneumonic	Pneumonic	Total	Non-pneumonic	Pneumonic	Total
Pneumococci	11 (5)	28 (9)	39 (14)	15 (5)	27 (6)	42 (9)	26 (8)	55 (15)	81 (23)
β-streptococci	2	2 (1)	4 (1)	3	3	6	5	5 (1)	10 (1)
Staph. aureus	—	2 (1)	2 (1)	—	13	13	—	15 (1)	15 (1)
H. influenzae	—	9	9	1	8	9	1	17	18
E. coli	1	3	4	4	6	10	5	9	14
Total	14	44	58	23	57	80	37	101	138
No. of cases	21	57	78	28	54	82	49	111	160

Table VI. Incidences of bacteria alone, antibacterial reactions alone, and bacteria together with serologic reaction against the same. Groups I and II are combined. Pneumonic versus non-pneumonic cases are reported separately, as well as patients not treated with antibacterial agents prior to admission versus those treated. Bacterial findings after the first week not counted. Isolations in all other cases of acute respiratory illness admitted during the observation time are included for comparison

Finding		Pnc	Pnc + APn	APn	Str	Str + AS	AS	Aur	Aur + ASta	ASta	H.I	H.I + AHI	AHI	E.c	E.c + ACol	ACol
Pneumonic	(111)	14	31	24	4	0	5	13	8	7	12	15	2	9	6	2
Non-pneumonic	(49)	4	12	14	1	1	4	4	0	0	2	1	0	2	3	3
Not treated	(114)	13	38	23	4	0	6	12	5	7	12	8		8	5	3
Treated	(46)	5	5	15	1	1	3	5	3	0	2	8	0	3	4	2
Total	(160)	18	43	38	5	1	9	17	8	7	14	16	2	11	9	5
Isolations		61			6			25			30			20		
Others	(914)	177			17			80			66			74		

Reactions against three different bacterial species occurred in four cases, against two species in 20 cases. In all, antibacterial reactions were recorded in 111 cases (69 %).

A report of the occurrence of bacteria, of serologic reactions against bacteria and of instances where both a bacterium and the corresponding antibody reaction were found is given in table VI. It can be noted that the various bacteria were recovered about twice as often in the investigated groups as in other patients not selected for this investigation. This may be due to an overrepresentation of cases with severe illness, such as pneumonia, or to the more intense diagnostic work devoted to the investigated groups.

Table VII. Incidence of Gram-positive versus Gram-negative bacteria in patients not treated with antibacterial agents prior to admission, in those treated with penicillin, and in patients treated with "broad spectrum antibiotics" and/or sulpha-compounds

Treatment	No. of pat.	Gram-positives	Gram-negatives	Pat. without findings
None.	114	72	33	36
Penicillin	33	11	13	12
Broad spectrum.	13	9	4	7

Table VIII. Serial number of the day after admission on which bacteria of different species were demonstrated for the first time. Isolations made after the first week are included. Patients treated with penicillin during the stay in hospital are reported separately from others (treated with "broad spectrum antibiotics" and/or sulpha-compounds, or not treated at all)

	Treatment					
	Penicillin-treated (79)			Others (81)		
	Day for first isolation					
	1–2	3–7	7–	1–2	3–7	7–
Pneumococci	32	3	0	15	9	1
β-streptococci	3	1	0	2	0	0
Staph. aureus (pen-sensit.)	13	0	0	7	1	0
Total	48	6	0	24	10	1
Staph. aureus (pen-resist.)	1	4	2	0	0	1
H. influenzae	5	6	1	17	2	0
E. coli	2	6	1	10	2	2
Klebsiella	1	1	2	3	0	0
Pseudomonas	0	0	1	0	0	0
Total	9	17	7	30	4	4
Total of both therapy groups	Penicillin-sensitive			72	16	1
	Penicillin-resistant			39	21	11

Further only 77 of 142 bacterial findings were accompanied by immune responses while, on the other hand 61 such responses were found in the absence of the corresponding bacterium.

The predominance of pneumococci and APn reactions is striking. However in 46 patients treated with antibiotics (generally penicillin) prior to admission only 10 isolations of pneumococci were made, as against 51 in the 142 non-treated ones ($0.02 > P > 0.01$ in Chi square test).

Staphylococci were found more often in pneumonic patients (21/111) than in non-pneumonic ones (4/49) but this difference is not significant. ASta reactions were found only in pneumonic patients and mainly in group II, that is during the influenza epidemic (13/54 as against 2/57 $0.01 > P > 0.001$) though no ASta reaction was observed in any of

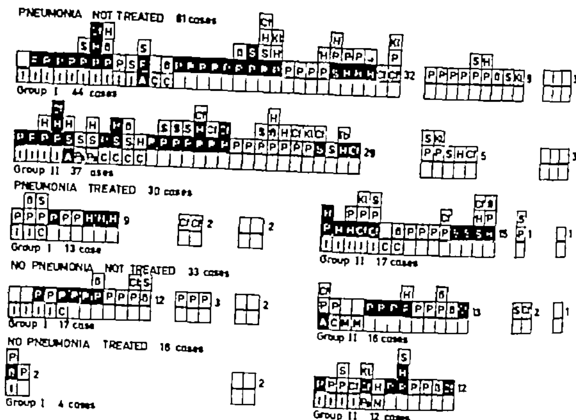


Fig 2. Isolations of viruses and bacteria, and significant antibody titre changes in the entire material of 160 patients with acute respiratory illness. Every patient is represented by a vertical set of signs. Virologic findings below the double line bacteriologic findings above.

White sign in black field = isolation + significant titre change
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A = adenovirus
I = influenza virus
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the few cases with a titre rise against influenza virus.

H. influenzae behaved similarly being found 27 times in 111 pneumonic patients but only three times in 49 non-pneumonic ones (0 01 > P > 0 001) AHI reactions were recorded in 17 pneumonic patients but in one only of the others (0 05 > P > 0 02 with Yates correction)

If it is sought to demonstrate the presence of bacteria as well as antibody responses, both the time elapsed from onset of disease to the first sampling, and antibacterial treatment given before admission are likely to be important. In table VII the incidences of Gram-positive versus negative bacteria are given together with the type of treatment *before* admission The total incidence was similar irrespective of treatment but apparently penicillin treatment effected a shift towards Gram-negatives. Of 24 strains

isolated from penicillin-treated patients 13 belong to this group, as against 33 of 105 from those not treated (0 05 > P > 0 02) Similarly among the 34 treated patients 14 out of 41 serologic responses were directed against Gram-negatives, as against 18 out of 97 in those not treated (0.05 > P > 0 02)

A similar effect of treatment *during* the stay in hospital is borne out by table VIII. From the third day of hospitalization penicillin-resistant organisms emerged 24 times in 79 penicillin-treated patients as against 8 times in 81 otherwise treated (0.01 > P > 0.001)

Mixed infection

In fig 2, an attempt has been made to collate the numerous observations in the material. Fresh infections with viral and/or bacterial agents, as judged from the serologic results, were found in 124 cases (77 %) potentially pathogenic bacteria only without any serologic reaction against the same, in 22 cases (14 %) and no such findings in 14 cases (9 %) These proportions were similar among pneumonic patients and non-pneumonic ones and also in those treated with antibiotics prior to admission when compared with those not treated. There was no special coincidence between any single virus and any single bacterial species.

Among the 124 sero-positive cases 17 had reactions against virus or mycoplasma only 37 also against bacteria (74 % of all cases with fresh virus infection) and 74 against bacteria alone.

The complexity of the pattern is well illustrated by the fig Serological evidence of fresh infection with *four* different agents, viral or bacterial was obtained in two cases, with *three* in eight cases, and with *two* in 42 cases, as against 72 cases with *one* agent only

Discussion

The material consisted of hospitalized patients only which means that a selection has already taken place. Except when sociomedical factors interfere, this selection favours conditions severe enough to cause hospitalization and complicating factors as for example advanced age, heart disease, chronic respiratory disease or diabetes. Furthermore, an overrepresentation of aetiologic agents resistant to antibiotics given at home is likely

The isolation of a potentially pathogenic agent in a case of acute respiratory illness does not in itself prove a causal significance of this agent. By the demonstration of a significant change in titre of an antibody against the agent a stronger time correlation between infection and disease may be established. This is still not equivalent to a causal relationship but makes it at least possible. Such considerations have been expressed in connection with many virologic studies (e. g 21) but have been largely neglected in bacteriologic work. Serologic methods have in fact seldom been employed in the study of bacteria in respiratory disease. This lack is the more obvious as bacteria potentially pathogenic for the respiratory tract may exist as apparently harmless inhabitants of the nose and throat, which fact makes the evaluation of their pathogenetic significance very difficult. Though higher titre averages have been found for AS (12) and ASta (16) among healthy carriers than in non-carriers of β-streptococci and staphylococci, a significant titre rise in a short time is likely to indicate an antigenic influence so effective as to be possible only when an inflammatory process is established. Therefore, we have considered changes in antibody titres as suggesting aetiological significance but bacterial isolations without serological

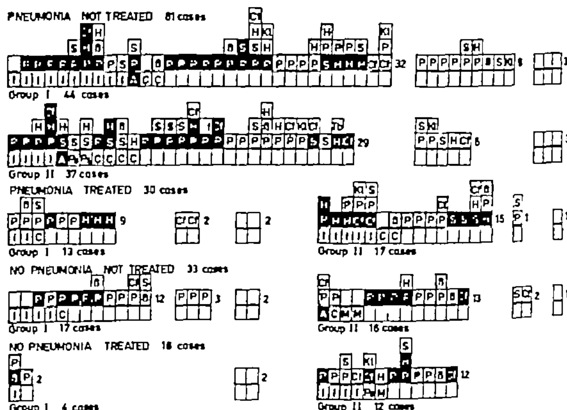


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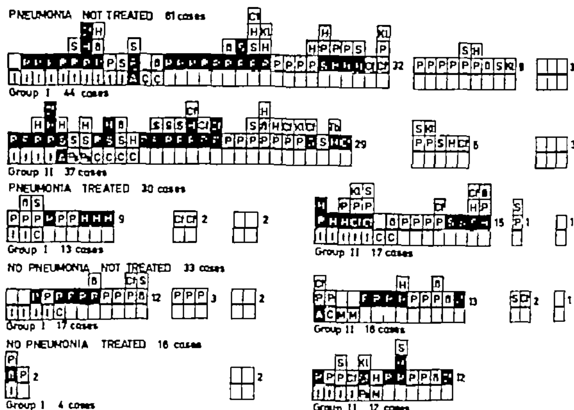


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If it is sought to demonstrate the presence of bacteria as well as antibody responses, both the time elapsed from onset of disease to the first sampling and antibacterial treatment given before admission are likely to be important. In table VII the incidences of Gram-positive versus negative bacteria are given together with the type of treatment *before* admission. The total incidence was similar irrespective of treatment but apparently penicillin treatment effected a shift towards Gram-negatives. Of 24 strains

isolated from penicillin-treated patients 15 belong to this group, as against 33 of 105 from those not treated ($0.03 > P > 0.02$). Similarly among the 34 treated patients 14 out of 41 serologic responses were directed against Gram-negatives, as against 18 out of 97 in those not treated ($0.05 > P > 0.02$).

A similar effect of treatment during the stay in hospital is borne out by table VIII. From the third day of hospitalization penicillin-resistant organisms emerged 24 times in 79 penicillin-treated patients as against 6 times in 81 otherwise treated ($0.01 > P > 0.001$).

Mixed infections

In fig. 2, an attempt has been made to collate the numerous observations in the material. Fresh infections with viral and/or bacterial agents, as judged from the serologic results, were found in 124 cases (77 %), potentially pathogenic bacteria only without any serologic reaction against the same, in 22 cases (14 %) and no such findings in 14 cases (9 %). These proportions were similar among pneumonic patients and non-pneumonic ones and also in those treated with antibiotics prior to admission when compared with those not treated. There was no special coincidence between any single virus and any single bacterial species.

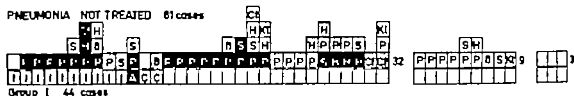
Among the 124 sero-positive cases 17 had reactions against virus or mycoplasma only, 37 also against bacteria (74 % of all cases with fresh virus infection) and 74 against bacteria alone.

The complexity of the pattern is well illustrated by the fig. Serological evidence of fresh infection with *four* different agents, viral or bacterial, was obtained in two cases, with *three* in eight cases, and with *two* in 42 cases, as against 72 cases with *one* agent only.

Discussion

The material consisted of hospitalized patients only, which means that a selection has already taken place. Except when sociomedical factors interfere, this selection favours conditions severe enough to cause hospitalization and complicating factors as for example advanced age, heart disease, chronic respiratory disease or diabetes. Furthermore, an overrepresentation of aetiologic agents resistant to antibiotics given at home is likely.

The isolation of a potentially pathogenic agent in a case of acute respiratory illness does not in itself prove a causal significance of this agent. By the demonstration of a significant change in titre of an antibody against the agent a stronger time correlation between infection and disease may be established. This is still not equivalent to a causal relationship but makes it at least possible. Such considerations have been expressed in connection with many virologic studies (e. g. 21) but have been largely neglected in bacteriologic work. Serologic methods have in fact seldom been employed in the study of bacteria in respiratory disease. This lack is the more obvious as bacteria potentially pathogenic for the respiratory tract may exist as apparently harmless inhabitants of the nose and throat, which fact makes the evaluation of their pathogenetic significance very difficult. Though higher titre averages have been found for AS (12) and ASta (16) among healthy carriers than in non-carriers of β-streptococci and staphylococci, a significant titre rise in a short time is likely to indicate an antigenic influence so effective as to be possible only when an inflammatory process is established. Therefore, we have considered changes in antibody titres as suggesting aetiological significance but bacterial isolations without serological









NO PNEUMONIA TREATED 16 cases

2 2 12

Group I 4 cases Group II 12 cases

Fig 2. Isolations of viruses and bacteria, and significant antibody titre changes in the entire material of 160 patients with acute respiratory illness. Every patient is represented by a vertical set of signs. Virologic findings below the double line, bacteriologic findings above.

White sign in black field = isolation + significant titre change.
Black sign in shaded field = significant titre change alone.
Black sign in white field = isolation alone.

A = adenovirus
I = influenza virus
Ps = psittacosis
C = cold agglutinins
M = Paul-Bunnell's reaction
P = pneumococci
β = β-streptococci
S = Staph. aureus
H = H. influenzae
Cf = coliform bacteria
Kl = Klebsiella
Tb = tuberculosis bacilli

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volved and no other aetiological factors are known.

The fact that in many cases the titre of one antibody has increased while the amounts of other antibodies have remained unchanged or diminished argues for a good specificity of the reactions employed and against a major significance of so-called anamnestic reactions in this type of material. Therefore, it seems evident that mixed infections are common in acute respiratory disease. But it cannot be decided to what extent more than one of the observed infections have been of pathogenetic significance for the disease, the others being simply coincidental. It should be recalled that many of the infections studied may occur without symptoms.

When viral and bacterial infections are observed together the latter are usually regarded as secondary complications precipitated by the virus infection and causing a more severe disease. The validity of this thesis cannot be judged from the present material.

In view of the complex pattern of potentially pathogenic agents found in this material and of the possibility demonstrated for a definition of this pattern, it seems reasonable to urge that this possibility be exploited in connection with therapeutic tests in this type of disease. However the difficulty of evaluating the significance of potentially pathogenic agents must be stressed.

Summary

One hundred and sixty cases of acute respiratory illness with or without bronchopneumonia have been analyzed by virologic and bacteriologic techniques including serological methods. It was thereby possible to demonstrate fresh infections with virus and/or bacteria in about 75 per cent of the material (mixed infection in 32 per cent.)

Pneumococci and serological reactions against pneumococci dominated in the bacteriologi study. Staphylococci and influenza bacilli as well as serologic reactions against these species were especially common among pneumonic patients. The elevations in titre of antibodies against bacteria were often transient, which resulted in frequent observations of titre decreases even within an observation time of one month.

Penicillin treatment prior to or during the hospitalization did not reduce the number of bacterial isolations but caused a shift towards Gram-negative organisms.

As the pattern of potentially pathogenic agents found and serologically shown to represent fresh infections was extremely complicated, sometimes involving three or four agents one of which was viral in nature, it seems reasonable to advocate a thorough analysis of materials of this type that are used for therapeutic studies. From the results obtained in the present study such an analysis can be achieved by use of available cultural and serological methods.

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responses as not informative in this respect. The extent to which these considerations have influenced the interpretation of our findings is illustrated by the fact that of 142 bacterial isolations and 138 titre changes in the present material only 77 coincided However this poor agreement between isolations and immune responses may be partly due to methodologic factors, as will now be discussed

First, if the blood samples are obtained too late in the course of disease or they are inadequately spaced a titre rise can be hidden Second the efficiency of the serological tests employed varies. As to the antilysin tests, the serologic response to an infection depends not only on the intensity of the inflammatory reaction and the efficiency of the antigenic stimulus thereby exerted but also on the ability of the bacterium to produce haemolysin This production can vary somewhat between streptococcal strains, more so among staphylococcal and pneumococcal ones, and most profoundly between strains of E. coli some of which do not produce any haemolysin at all. In the case of the complement fixation test for H influenzae antibody though the antigen has been chosen so as to contain factors widely represented among different strains of the species, the degree of coincidence between these factors and those of the infecting strains must vary from one case to another Similar limitations apply to some of the nonspecific reactions and those against viruses, as for example the cold agglutination test which has been found positive in about 50 per cent only of infections with Eaton s agent, although probably more often in cases with pneumonia Similarly some infants with influenza may not develop recognizable levels of antibody against soluble antigen.

For these reasons and provided that so-called anamnestic reactions provoked by heterologous stimuli do not play any major part in this material the serologic reactions are likely to give only minimum figures for the actual immunological responses.

On the other hand, no technique for the isolation of virus or bacteria yields a total recovery of all agents present. Further antibiotic treatment before admission may eliminate all possibility of isolating sensitive bacteria which have already elicited an immunological reaction.

The significance of titre decreases has been discussed in a preceding paragraph. These decreases, often observed in bacterial infections may be partly due to the fact that antibiotic treatment may make the antibody response weak and transient, as Ström (24) has shown in scarlet fever

A predominance of pneumococci and serological reactions against pneumococci in all types of acute respiratory illness has been demonstrated in the present material. Staphylococci and H. influenzae as well as immunological responses against them were most common in pneumonic cases.

In cases treated with penicillin prior to admission the recovery of bacteria and detection of serologic reactions against them was as common as in patients not treated, though a shift towards Gram-negative penicillin resistant organisms took place. Also during the stay in hospital penicillin treatment resulted in the emergence of penicillin resistant organisms and serologic reactions against them. These observations make the value of penicillin therapy seem questionable in respiratory infections at home as well as in hospital when viral infection is likely to be in-

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Basophil and Eosinophil Leukocytes in Various Internal Disorders

By

Lennart Juhlin

A white blood cell differential based on 200 white cells usually shows between 0—2 per cent basophil leukocytes. Increased numbers of these cells have been reported in chronic myelogenous leukaemia and polycythaemia vera. With the introduction of the direct method of counting (23) more exact information about the normal number of basophils became available. A decrease in the basophil cell content of the blood has been described mainly in connection with stress, thyrotoxicosis, and urticaria. Our present knowledge of the basophils under various conditions has recently been reviewed by Braunsteiner and Zucker Franklin (8) Rorsman (28) and Thonnard Neumann (32)

Human basophils contain both heparin and histamine. This suggests that the cells might be of importance in allergic reactions, blood coagulation, fat metabolism and arteriosclerosis (30) A new method (29) now makes it possible to study both the number and morphology of the basophils. According to this the basophils are divided into 18 different types depending on the number size and distribution of their granules. The frequency of these types in the blood gives a basophil differential. In the present paper the method has been used to study the basophils in some common internal disorders.

Submitted for publication February 18, 1963.

Methods

Counting and examination of basophils in blood

The method for handling the basophils has been described elsewhere in detail (29) In principle, venous blood is withdrawn into a siliconized needle and syringe and forcibly ejected into a cold fixative (acetic acid 20, ethyl alcohol 60, chloroform 20) This destroys all of the erythrocytes, leaving only the leukocytes. The white cell suspension is filtered through coarse membrane filter of cellulose (Cella 0) The cells remain on the filter where they are stained with toluidine blue. The filter is then dehydrated in ethyl alcohol, cleared in xylene, and mounted on a microscope slide. It is then possible to study the morphology of the basophils, which appear as distinct cells with specific, dark metachromatic staining of the granules. The other white cells are pale blue with a faint greenish cast to the granules of the eosinophils

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Basophil and Eosinophil Leukocytes in Various Internal Disorders

By

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The method for handling the basophils has been described elsewhere in detail (29). In principle, venous blood is withdrawn into a siliconized needle and syringe and forcibly ejected into a cold fixative (acetic acid 20, ethyl alcohol 60, chloroform 20) This destroys all of the erythrocytes, leaving only the leukocytes. The white cell suspension is filtered through a coarse membrane filter of cellulose (Cella 0) The cells remain on the filter where they are stained with toluidine blue. The filter is then dehydrated in ethyl alcohol, cleared in xylene, and mounted on a microscope slide. It is then possible to study the morphology of the basophils, which appear as distinct cells with specific, dark metachromatic staining of the granules. The other white cells are pale blue with a faint greenish cast to the granules of the eosinophils.

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Table I Basophil, eosinophil and total white blood cell counts in various disorders. The figures indicate the mean ± S E of mean

Diagnosis	No. of pat.	Basophils (/mm³)	Eosinophils (/mm³)	WBC
Controls	25	35 ± 3	171 ± 6	6,000
Bronchial asthma	10	33 ± 5	564 ± 1 6	7,700
Diabetes (insulin-treated)	10	31 ± 5	143 ± 56	6,100
Thyrotoxicosis	10	17 ± 5	111 ± 38	5,100
Myxoedema	5	60 ± 12	180 ± 50	6,000
Pernicious anaemia	7	31 ± 7	193 ± 38	6,200
Essential hypertension	6	24 ± 5	136 ± 25	7,600
Ulcerative colitis	10	74 ± 11	592 ± 231	11,500
Bleeding peptic ulcer (verified by X ray)	8	14 ± 4	157 ± 51	6,200
Bleeding gastric ulcer (not detected radiologically)	9	32 ± 6	193 ± 48	6,000

Under a magnification of 640 × 20 or 40 consecutive basophils were classified into three major groups A, B and C, depending upon the size of the granules and the depth of the staining Furthermore, each group is subdivided into six classes (1 to 6) according to the number and location of the granules.

The percentage of basophils was estimated by counting the number of all the white cells seen while finding the 20 or 40 basophils. The total number of basophils has been estimated by simultaneously counting the number of white cells/mm

Eosinophils

The total number of eosinophils was counted in the manner described by Thorn et al. (33)

General procedure

The blood specimens were always taken before breakfast between 8 and 8.30 a. m. None of the patients was receiving corticosteroid therapy or had recently had urticaria.

Results

The results are summarized in table I In connection with this the following points may be noted.

1 *Bronchial asthma*

The patients were treated with theophylline derivatives and sedatives. They had a normal mean number of basophils and a normal basophil differential. Their eosinophil counts varied widely The individual values of both types of cells appear in fig 1 No correlation could be established between the number of basophils and eosinophils.

2 *Diabetes mellitus*

Three patients with insulin coma had a decreased number of basophils (3 12, 14/mm) Three untreated diabetics with signs of prediabetic coma had normal basophil levels (30 40 and 60/mm³) During treatment with insulin there was a temporary increase in their basophil count. Diabetic patients under satisfactory control with insulin had a normal basophil count. The individual values of the cells are plotted together with the eosinophils in fig 1 No correlation was found between the number or type of basophils and the blood sugar level.

3 *Thyrotoxicosis*

The total number of basophils was below normal. The basophil differential was normal and the cells did not show any signs of degranulation. The patients basal metabolic rate (BMR) varied be-

tween + 35 and + 82 per cent. No correlation was found between the BMR and the basophil count.

4. *Myxoedema*

The basophil count was high. Since only 5 patients were investigated the divergence is not significant.

5. *Ulcerative colitis*

The total number of basophils was slightly above normal. Details of these results have been presented elsewhere (16).

6 *Peptic ulcer*

In acute bleeding peptic ulcer which could be verified radiologically the basophil leukocytes were decreased and showed signs of degranulation. In patients with the same symptoms but where no ulcer could be detected by X-ray examination the basophils were normal in number and appearance (17)

7 *Pernicious anaemia*

The patients had previously been treated with vitamin B_{12}. They were admitted to the wards for special studies as soon as their blood level of vitamin B_{12} became subnormal. Normal basophil counts and differentials were found both before and after repeated treatments with vitamin B_{12}.

8. *Essential hypertension*

The 6 patients investigated were between 30 and 45 years old. Their basophils were within normal limits with the exception of one patient, who at repeated examinations had between 2 and 9 basophils/mm³ In 4 patients treated with 100 mg of guanethidine (Ismelin®) for 3 days the basophil count remained unchanged.

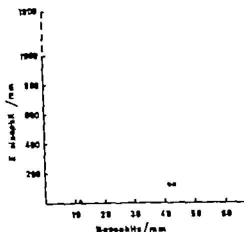


Fig 1 Number of eosinophil and basophil leukocytes in patients with bronchial asthma (○) and diabetes (●).

9 *Myocardial infarction*

In 15 patients admitted to the wards for acute myocardial infarction the basophil and eosinophil leukocytes were counted during the course of the disease. The patients were kept in bed for about 6 weeks. Thereafter they were allowed to sit up for gradually increasing periods, starting with 10 minutes a day When discharged around the seventh or eighth week they were sitting up for about 2 hours a day and allowed to move around carefully for 10—20 minutes. At home their physical activity was successively increased.

The drugs used for treatment varied. Patients under 70 years received anti prothrombin (Tabl. AP®) to decrease the tendency to blood coagulation. Procainamide (Pronestyl®) digitalis, sedatives, analgetics and vasodilating agents were given when indicated.

The mean numbers of basophil and eosinophil leukocytes on the days immediately following the onset of the disease are shown in fig 2. The basophils

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Diagnosis	No. of pat.	Basophils (/mm³)	Eosinophils (/mm³)	WBC
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3 *Thyrotoxicosis*

The total number of basophils was below normal. The basophil differential was normal and the cells did not show any signs of degranulation. The patients' basal metabolic rate (BMR) varied be-

between the numbers of the two cells ($P < 0.05$ at 7 14 and 42 days $P < 0.01$ at 21 28 and 35 days) No correlation was found 3 months after the infarction.

Discussion

Some authors describe an increase in basophil leukocytes in bronchial asthma (7) Others report normal basophil counts (25 26, 31) Rorsman (28) points out that the normal values he obtained might be due to the fact that most of his patients were receiving corticosteroids. Though none of the patients investigated here were under such treatment the mean numbers of their basophil and eosinophil leukocytes were normal, too. The eosinophils varied widely No correlation between the number of eosinophils and basophils was found Thus the assertion (7) that bronchial asthma is the only allergic disease where there is a close relationship between eosinophils and basophils could not be confirmed.

Diabetic patients whose condition was adequately controlled with insulin had normal basophil (31/mm³) and eosinophil counts. This is in agreement with Tedeschi et al. (31) and Engleson and Lindberg (11) who found normal basophil counts in diabetic patients. Braunsteiner et al. (6) investigating 20 diabetic patients, obtained a mean of 41.5 basophils/mm³ which was significantly above that of their normal subjects (28.1/mm³) The difference was slight, as pointed out by the authors, and only patients not under stress had been selected. I can offer no certain explanation for the discrepancy between these results and ours. Possibly the dose of insulin we used was higher and thus produced an unspecific stress reaction.

The increased basophil count found in myxoedema and the decreased count in thyrotoxicosis are in agreement with those of earlier investigators (7 9 14 15 21 22 31 34) The same is true of the basophil percentage found in pernicious anaemia (1) Treatment with vitamin B_{12} did not change the number or type of basophils.

The day after a myocardial infarction the basophils were degranulated Four weeks later the basophil differential had returned to normal. Usually the basophil count did not visibly decrease until 2—3 days after the heart attack, whereas the eosinophils dropped immediately A similar delay in the basophil response has been reported during corticosteroid treatment (18) In 2 patients studied before and during the heart attack normal basophils were found. This means that the basophil decrease is probably due to the stress reaction produced by the infarction. In patients with myocardiosclerosis Tedeschi et al. (31) reported normal basophil values, which is in agreement with our findings.

From 7 to 35 days after the infarction the ratio between the number of basophil and eosinophil leukocytes was fairly constant for each patient. When all the individual values are plotted in a diagram (fig 3) there is a significant correlation between the two cell types. This seems to be due to the presence of a group of six patients with an abnormally high number of eosinophils and basophils. Among the patients with normal cell counts no correlation was obtained. In a previous investigation with cases of ulcerative colitis (16) both extremely high and low eosinophil and basophil levels had been found, but no correlation between the cells. The difference between the heart cases and the others

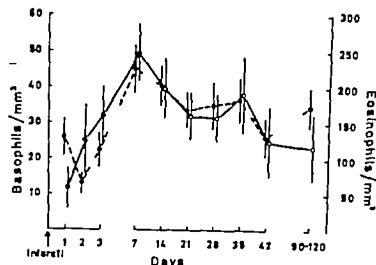


Fig 2. Mean number of circulating eosinophil and basophil leukocytes after myocardial infarction. Vertical lines indicate the standard error of the mean.
● ● Basophils (left ordinate).
○—○ Eosinophils (right ordinate).

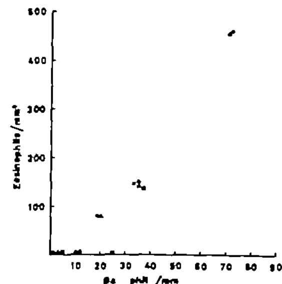


Fig 3. Number of circulating eosinophil and basophil leukocytes 7 (●) 14 (×) 21 (○) 28 (△) 35 (□) and 42 (▲) days after myocardial infarction.

did not decrease markedly until the second day after the heart attack. By the 7th day they had returned to normal. The eosinophils on the other hand decreased already the first day after the heart attack. They too regained their normal values within a week. During the next 7 days the tendency to increase was not significant in either basophils or eosinophils. Exceptionally patients showed a decrease in both types of cells when they were mobilized after six weeks. Concerning the mean values there was no significant decrease at this stage, however (fig 2)

The first days and weeks after the infarction the basophils showed signs of degranulation Types $A_{1-3} + B_{1-3}$ decreased ($P < 0\ 01$) and groups 4 + 5 increased ($P < 0\ 001$) By the 4th to 5th week after the onset of the disease the basophil differential had gradullay returned to normal.

In 2 patients who suddenly died after 3 and 4 weeks the basophils were studied 4 and 24 hours before death, respectively In one of these patients a blood sample was also taken when cardiac puncture was made in a fruitless attempt to reanimate the heart when it stopped. These specimens showed the same normal number and types of basophils as the foregoing week.

During the weeks following the infarction there was often a close correlation between the number of basophil and eosinophil leukocytes. The values obtained 7—35 days after the heart attack are shown in fig 3 It is evident that in the absence of exertion there is a correlation

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might be due to the fact that the patients with myocardial infarction were more homogeneous and that all of them were confined to bed. A correlation between the number of circulating eosinophils and basophils has been reported by some investigators (10 13 20 24 27) Others failed to find any correlation (3 5 6 7, 21 30) In guinea pigs sensitized with egg albumin, Winquist (36) obtained a marked increase in both basophil and eosinophil leukocytes. Since the basophil is the circulating histamine cell (12) and the eosinophils supposed to be the anti histamine cells (4 19 35) a correlation seems probable under certain circum stances. Where there is none it may be suspected that one of the cell types has been destroyed or trapped in the tissues or that its production has decreased or increased.

Summary

The number and morphology of the circulating basophil leukocytes have been studied in some common internal diseases. The eosinophil leukocytes were counted at the same time. Normal numbers and types of basophils were found in patients with bronchial asthma, essential hyper tension diabetes mellitus and pernicious anaemia. The basophil count was low in thyrotoxicosis and probably high in myxoedema. Two to three days after a myocardial infarction the basophils were decreased and degranulated The count returned to normal within a week, and the signs of degranulation gradually dis appeared within a month. The decrease in basophils might be explained by the stress reaction caused by the cardiac in- farction. A positive correlation between the number of eosinophils and basophils could be established 1—6 weeks after the infarction. In healthy subjects and the other disorders studied no correlation be- tween the cells was found. The findings are discussed and compared with other investigations.

Acknowledgements

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The Pigmentation of Thyrotoxic Patients

By

K. Kirkeby G Hangaard and P Lindjærde

Excessive cutaneous pigmentation, diffuse or localized, is present in some cases of thyrotoxicosis. Pigmentation of the creases of the palms, in scars or in the periorbital region may be found. The pathogenesis of this pigmentation is unknown.

It is generally believed that the cause of the increased pigmentation in Addison's disease is a high blood concentration of ACTH, which has a structural similarity with the pigment hormone.

We have studied cortisol metabolism in thyrotoxic patients and the findings we have made have led us to the hypothesis that the cause of the pathologic pigmentation in this disease may be the same as in Addison's disease — a high blood level of ACTH.

Material and methods

Six pigmented and six unpigmented thyrotoxic patients were studied and compared with control group of twelve healthy subjects. The criteria for thyrotoxicosis included the classic symptoms and signs of the disease. The BMR ranged between + 25 % and + 86 The criterion for putting the patients in the pigmented group was definite localized pigmentation — in scars, in creases of the palms or periorbitally

After an infusion of cortisol (1 mg/kg body weight in 250 ml 5 % glucose, given over a 20-minute period) blood samples were drawn for determination of free 17-hydroxy corticosteroids in plasma at one-half, one, two, four and six hours. The level of free 17 hydroxycorticosteroids was measured by the technique of Peterson et al. (10)

Results

The results are given in fig 1 representing the average values obtained in the pigmented and in the unpigmented thyrotoxic patients, respectively and in the control group and in table I which records the values in the thyrotoxic subjects.

In both thyrotoxic groups the disappearance rate of the administered cortisol was *greater than in a* group of control patients. In the first hours of th test no difference in the disappearance of 17-hydroxycorticosteroids was found between the two groups of thyrotoxic patients At the end of the test, however

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Book reviews

Erkrankungen der Leber und der Gallenwege By I. Magyar Vol. 2 351 pp 34 ill. 8 tab Price Ft 320 Verlag der Ungarischen Akademie der Wissenschaften Budapest. Akademie-Verlag, Berlin 1961

The second part of this handbook deals chiefly with cirrhosis of the liver diseases of the hepatic vessels, bile ducts, gall bladder and the reaction of the liver in common diseases and other not directly hepatogenic pathological conditions.

The book has great merits and the author presents his experiences side by side with those of other researchers back into historical times. In the extensive bibliography after each section, however one lacks a number of references from recent years, though this is to a great extent made up for by the author's own thorough knowledge of the field.

The typographical arrangement leaves something to be desired and likewise one would wish for a larger number of illustrations. These objections, however are relatively unimportant, and the work can undoubtedly be recommended as a book of reference for liver diseases. The references to the literature are abundant up to a few years back, and in them the reader will readily find sources of information on points of detail.

Lars Lindgren
Stockholm

Krankheiten der Leber und der Gallenwege in der Praxis By N Markoff and E Kaiser 407 pp 258 ill. some in colour 55 tab. Price DM 69 — Georg Thieme Verlag, Stuttgart 1962.

The book starts with an account of the anatomy physiology pathophysiology and blood chemistry of the liver The chapters on general diagnostics contain full accounts of laboratory practices with an assessment of the diagnostic value of each test. For the evaluation of the pathoanatomical picture in vivo there are technically very fine and instructive colour photographs, taken in the course of laparoscopy and for the collection of histological specimens a detailed account is given of Menghini's technique as applied to liver biopsy The modern methods of X ray examination are illustrated by pictures of such a kind and quality that even a non-specialist can easily interpret them.

The main emphasis is on the description of virtually all medical and surgical ailments of the liver and biliary tract, and on their treatment. These lucid and carefully reasoned chapters include data on differential diagnosis, arranged in an easily surveyable form and well adapted for teaching, often in the shape of instructive tables.

The clear arrangement throughout and the easy-flowing language, not to mention the first-class illustrations, make the book pleasant reading At the same time one is surprised that, in spite of the comparatively small number of pages, the authors have succeeded in including so much of the variegated flora of the liver diseases. A fairly extensive bibliography listing chiefly the literature from recent years, provides a signpost for those who wish to penetrate into details. As advanced textbook and book of reference it is warmly recommended.

Lars Lindgren
Stockholm

patients are more conflicting, but in some cases hyperplasia of the adrenal cortex has been demonstrated (5).

The hyperactivity of the adrenal cortex in thyrotoxicosis most likely is caused by the regulatory mechanism of the anterior pituitary — with a high production of ACTH as a consequence of and a compensation to the rapid degradation of cortisol.

The results seen in the present experiments might well be obtained in patients chosen so that the pigmented group was slightly more hyperthyroid than the unpigmented group. In our study the reverse was true. The mean value of BMR was slightly higher in the unpigmented than in the pigmented group.

Thus, this study seems to indicate a difference in cortisol metabolism between the two groups. The very low levels of free 17-hydroxycorticosteroids found in the pigmented patients 6 hours after the infusion of cortisol are of a magnitude which could provoke the regulatory and compensatory mechanism of the hypothalamus and the anterior pituitary. It is possible that the increased pigmentation in thyrotoxicosis thus may be caused by a high concentration of ACTH, as in Addison disease — owing not to adrenocortical *insufficiency but to* the rapid degradation of cortisol.

Our study was done four years ago. The previous hypothesis about increased ACTH secretion in thyrotoxic patients recently has been confirmed more directly with methods based on the isolated perfused adrenal gland preparation of hypophysectomized dogs (4).

Summary

It is known that the degradation of cortisol is accelerated in thyrotoxicosis.

The present study has demonstrated a more rapid disappearance of cortisol from plasma in pigmented than in unpigmented thyrotoxic patients.

It is suggested that the pathologic pigmentation occasionally observed in thyrotoxicosis is caused by an increase in ACTH production, as a consequence of and a compensation for the accelerated degradation.

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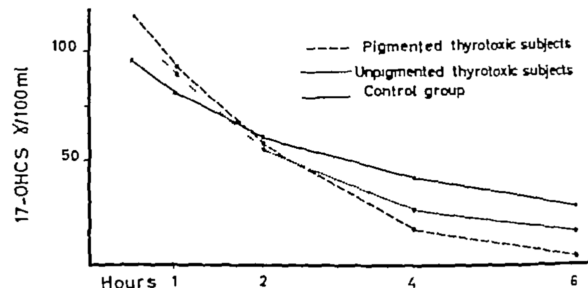


Fig 1 Cortisol degradation in pigmented and unpigmented thyrotoxic patients.

Table I Cortisol degradation in pigmented and unpigmented thyrotoxic patients Cortisol in plasma in µg/100 ml

	Hours					
	0	/	1	2	4	6
Pigmented						
1	12.3	112.3	101 1	62.4	23.3	4.6
2	24.9	146.9	94.8	69.3	4 0	0.9
3	12.6	158.6	135.9	85.4	30.7	2.0
4	15.1	97.9	68.9	37.0	4 4	0
5	9.3	86.4	66.9	36.3	11 1	7.5
6	14 7	99.0	85.9	54.7	24.5	3.8
Mean	14.8	116.9	92.2	57.5	16.3	3 1
Unpigmented						
1	21 1	95.2	73.4	41.0	31.2	27 4
2	23.0	129.2	107 7	67.3	16.8	11.5
3	21.2	97.6	74.3	37.8	28.7	12.9
4	12.0	129.3	113.1	65 4	30.5	7.8
5	15.6	96.2	74.8	59.7	15 3	16.1
6	15.9	116.8	99.2	79 4	32.1	14.9
Mean	18.1	110.7	90 4	55 1	27 4	15.1

there was a definite tendency towards lower values in the pigmented group The mean level in these patients at 6 hours was 3 1 µg/100 ml (range 0—7.5) compared with a mean value of 15 1 µg/100 ml in the unpigmented patients (range 7 8—27.8) The difference between the means is statistically significant with a p less than 0 005 when evaluated statistically by a t test

Discussion

The most unequivocal finding concerning adrenocortical hormones in thyrotoxicosis is a more rapid degradation of cortisol than in normal subjects (3 7 9) However essentially normal values of 17 hydroxycorticosteroids in blood are found and definite symptoms of adrenocortical insufficiency usually are lacking Consequently the adrenal cortex of thyrotoxic patients must be overactive in its cortisol production. Experimental support for this comes from the finding of high basal excretion of urinary 17 hydroxycorticosteroids (2) Histopathological examinations of animals made thyrotoxic by thyroxine show hyperplasia of the adrenal gland (1 6 8) The findings at autopsy in thyrotoxic

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A Thalassaemic Trait in Gipsies?

By

H. Zilliacus and A. M. Ottelin

Abnormal haemoglobins have been found to occur among a variety of mediterranean people. The occurrence of foetal haemoglobin (Hb F) after the second year of life is regarded as abnormal. The persistence of Hb F during adult life is found among others in thalassaemias. In the gravest form of this, in Cooley's anaemia or thalassaemia major there is a high percentage of Hb F (2) Varying quantities of Hb F are found in anaemias of thalassaemia minor (5) and minima (6)

Method

The staining method described by Kleihauer and Betke (4) has proved very suitable for identification of Hb F in the erythrocytes. As compared with the alkali denaturation technique, the method until now most commonly used for estimation of Hb F (3 7), the staining method has the advantage of being quick and sensitive even to very small amounts of Hb F

Hb A, precipitated by action of drying and 80 ethanol becomes easily soluble in citric acid phosphate buffer pH 3.2—3.4 Precipitated Hb F is very slowly soluble under these conditions. After staining with acid haematoxylin and with erythrosine cells containing Hb F are deep red and cells containing Hb A, appearing as ghosts, are scarcely visible with oil immersion.

Results

When the staining technique described by Kleihauer and Betke (4) for visualisation of foetal haemoglobin in blood smears was applied to a series of children between the ages of one month and two years, it was found that a smear obtained from a nine-month-old gipsy boy was stained light pink throughout. In contrast to smears from normal children of the same age, in which a number of erythrocytes staining deep red are found among non-staining ghosts, nearly all the erythrocytes in the smear from the gipsy seemed to contain small amounts of red-staining substances. Microcytes, ovalocytes, target cells, and polychromasia were observed in non-stained smears and in smears stained with the May Grunwald-Giemsa technique. The liver and the spleen of the child were reported

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Hb A, precipitated by action of drying and 80 ethanol becomes readily soluble in citric acid phosphate buffer pH 3.2—3.4. Precipitated Hb F is very slowly soluble under these conditions. After staining with acid haematoxylin and with erythrosine cells containing Hb F are deep red and cells containing Hb A, appearing as ghosts, are scarcely visible with oil immersion.

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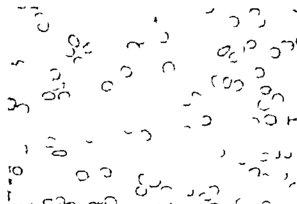


Fig 1 Blood smear from a nine-month-old gipsy boy Ovalocytosis, aniso- poikilocytosis, target cells.

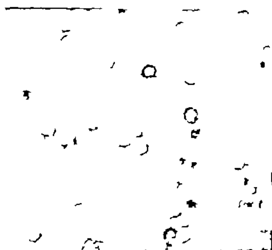


Fig 3. 30-year-old gipsy woman (same as No. III in fig 2) Visualisation of erythrocytes containing Hb F Kleihauer staining technique.

to be enlarged and occasional rises of temperature had occurred for no special reason. We now examined blood smears from the mother and the father of the child both gipsies, and from an additional three adult gipsies one of whom had the same surname as the gipsy child In all the smears examined some abnormal features were found either ovalocytes, elliptocytes, aniso- or poikilocytosis, schistocytes or a few target cells being present in varying combinations. Staining of the smears with the Kleihauer technique showed a light pink colouration of the erythrocytes of the mother's smear and a much more pronounced colouration of the erythrocytes in the smear obtained from the gipsy child's father In this smear among the light pink erythrocytes there were a number of "Kleihauer cells, fully stained erythrocytes. The picture thus obtained

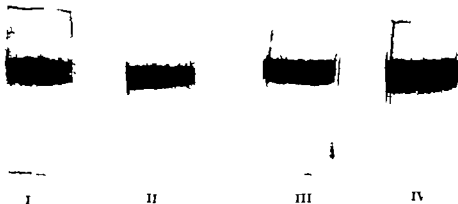


Fig 2. Electrophoresis on starch gel.
I Control, normal newborn, 3 460 g
II 9-month-old gipsy boy
III 30-year-old gipsy woman.
IV 41 year-old woman, normal.
Hb F (Hb A ?) distinguishable in I II and III.

closely resembled that described by Fraser and Raper (1) in cases of thalassaemia minor

Staining of blood smears for visualisation of Hb F obtained from the three other gipsies revealed varying degrees of red staining of the erythrocytes. In two of these cases most of the erythrocytes contained varying amounts of substances staining slightly pink. In smears from the third gipsy (a girl of 15 years) there were approximately 6 per cent of deep red stained "Kleihauer" cells, among normal adult ghosts" indicating that about 5—6 per cent of Hb F was present. Estimation of the foetal haemoglobin with the alkali denaturation technique developed by Jonxis and Visser (3) showed the amount of Hb F to be 6 1 per cent. There was thus a good conformity of the two methods for estimation of Hb F Osmotic fragility in NaCl was 0.78—0.36 %, RBC 3.38 (10^6/mm³) Hb 10.7 (g/100 ml) Colour index 0.99 WBC 8,600 Platelets 115,000 Reticulocytes 4 %. Haematocrit 31 % Serum iron 91 μ %

On starch gel electrophoresis in borat buffer the haemolysates from the gipsy child and a gipsy woman of 30 behaved like a control haemolysate from a normal newborn infant (3 400 g) there was a distinct separation of a fraction of the haemoglobin at the level of the foetal component of the control. The possibility remains, however that this fraction is not Hb F but haemoglobin of type A_2.

Discussion

Morphological abnormalities of the erythrocytes like those observed in the smears from gipsies described here, the occurrence of Hb F (possibly Hb A_2) and some other haematological anomalies indicate a thalassaemic trait. As there are to our knowledge no reports in the literature of thalassaemia among gipsies and no case of thalassaemia has been described in Finland, we thought this report to be of interest.

Taking into consideration that ethnologically the gipsies are believed to be descended from Indians in northern India where thalassaemia is frequent and that they have hardly interbred at all with the rest of the population in the countries they now inhabit, a closer study of this possible thalassaemia has most interesting aspects.

Summary

Morphological abnormalities of erythrocytes, the occurrence of foetal haemoglobin and certain laboratory findings speak in favour of a possible thalassaemic trait in six gipsies observed.

Acknowledgements

W are indebted to Prof. P Forsell, head of the children home, Folkhälsan, for permission to take blood samples from the gipsy child, and to Dr T Vainio, State Serum Institute for performing the test on the starch gel electrophoresis.

This work was supported by grant from the Sigrid Juselius Stiftelse.

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Cardiac Catheterization Studies Following Injection of Synadrin®

By

A. M Abrahamsen

Synadrin® N-[3' phenyl-propyl-(2')]-1 1-diphenyl-propyl (3)-amine, is a new coronary vasodilator drug In the isolated heart of guinea-pig Lindner (10) has found an average increase of 51 per cent in the coronary blood flow after intra venous injection of 10 μg Synadrin, while intracoronary injection of 1 mg Synadrin induced a short increase of 110 per cent. Following the intravenous injection of 0.8 —1 6 mg/kg in dogs, Kochsiek et al. (9) found that the coronary blood flow increased by 82 per cent, while the oxygen consumption of the heart at the same time increased by 18 per cent. The oxygen saturation in the coronary sinus blood rose from 19 to 41 per cent. Bohm et al. (4) found that in dogs oxygen transport increased considerably more than the simultaneous consumption of the myocardium.

Synadrin has sympatholytic and sedative effects (6, 10)

Numerous clinical trials (1 2 3 4 7 8, 14 15 16) have shown an improvement of the symptoms in diseases with coronary insufficiency

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The intention of this study is to examine the hemodynamic effect of Synadrin, especially on the pulmonary circulation The hemodynamic examinations are performed by cardiac catheterization, not used before in this connection.

Hemodynamic studies following injection of Synadrin have been previously carried out in animals and using another method, in human beings (2 5 6 10 11)

Garten (5) found a drop in the pulmonary capillary venous pressure following the injection of Synadrin in patients with mitral stenosis.

Material and methods

The material consists of seven patients with mitral stenosis, six women and one man. The ages varied between 37 and 57 years, average 43 years.

Right sided cardiac catheterization was performed with the patient in a supine position before breakfast. The pressures were recorded by means of an Elema strain gauge electromanometer and two-channelled direct writing electrocardiograph. The zero level was the fourth intercostal space in the anterior axil-

Table I Changes in mean pulmonary arterial pressure (PAP) pulmonary capillary venous pressure (PCV) blood pressure (BP) cardiac output (CO) heart rate stroke volume and calculated pulmonary vascular resistance before (a) and 60 minutes after (b) injection of 5 mg Synadrin® The students t-test is used for statistical calculation

Case	PAP (mm Hg)		PCV (mm Hg)		BP (mm Hg)		CO (l/min)		Heart rate (beats/min)		Stroke volume (ml)		Pulm. vasc. resist. (dyn. sec cm⁻⁵)	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
1	18	16	9	10	165 /80	160 /80	4.9	4.3	70	68	70	63	147	112
2	7	7	6	3	130 /80	130 /85	4.9	4 6	89	72	55	64	16	70
3	33	20	16	13	130 /90	125 /85	6.0	6.0	115	86	52	70	227	93
4	18	19	—	—	115 /75	105 /60	—	—	50	54	—	—	—	—
5	81	81	—	—	110 /75	105 /75	—	—	99	94	—	—	—	—
6	13	11	10	7	115 /80	105 /75	8.9	8.6	80	79	111	109	27	37
7	23	13	16	12	120 /80	115 /80	3.6	3.4	90	75	40	45	156	24
Average	27.6	23.9	11 4	9.0	126.4/80	120.7/77 1	5.66	5.38	84 7	75 4	65.6	70.2	114.6	67.2
M. diff	−3 7		−2 4		−5 71/2.86		−0.28		−9.3		+ 4 6		−47 4	
P	0.01 < P < 0.05				Syst.: 0.001 < P < 0.01		0.01 < P < 0.05							
t	1 78		2 75		Syst. 4.38		2.89		2.13		0.94		1.26	

lary line The pressures were recorded in the right atrium, pulmonary artery and in wedged position. The heart rate was recorded from the electrocardiogram The catheter was then withdrawn to the pulmonary artery and the pressure was recorded again. A Cournand needle was placed in the femoral artery and the cardiac output was estimated, using the Fick principle. 10 ml (5 mg) Synadrin, as gluconate diluted in distilled water to 20 ml was then injected through the catheter in the pulmonary artery within three minutes. The pressure recordings were repeated after 5 10, 20, 30 40, 50 and 60 minutes. Simultaneously the brachial artery pressure was controlled by a cuff. A second determination of cardiac out put and pulmonary capillary venous pressure was done about 60 minutes after the injection

Results

The circulatory effects 60 minutes after the injection of Synadrin are seen in table I

There was a slight, but not significant drop in the pressure in the pulmonary artery But a significant reduction of the pulmonary capillary venous pressure was recorded both maximum and minimum pressure, and also of the mean pulmonary capillary venous pressure.

The systolic systemic blood pressure was reduced significantly while there was an insignificant drop in the diastolic and mean arterial pressures.

The mean calculated pulmonary vascular resistance dropped from *114* to *67* dyn sec. cm⁵ but the reduction is not significant.

In six of the seven patients there was a reduction of the heart rate 60 minutes after injection of Synadrin the insignificant average reduction in all patients being 9 beats/min

In five of the patients the cardiac out put was examined before and after in-

jection of Synadrin and a slight, but significant reduction was found. The stroke volume and calculated total peripheral vascular resistance were not significantly changed.

During the trial no lengthening of the PQ interval or widening of the QRS complex was recorded.

No side effects of Synadrin were observed.

Discussion

In animal experiments Lindner (10) found an increase in the heart rate during the first three minutes after the injection. In humans Garten (5) found an increase and Brandschwede (2) a decrease in the heart rate 10—15 minutes after the injection. After 60 minutes most investigators (5 6, 11) have recorded a reduction of the heart rate as in this study

In animal studies the cardiac output increased during the first three minutes, returning later to control values (10) Using another method Michel (11) found an insignificant reduction of the cardiac output after 30 minutes in human beings, while in this study there was a significant reduction after 60 minutes.

In this study there was some reduction of the systemic blood pressure, while Michel (11) found practically no change. There was, as mentioned before, a significant reduction of the pulmonary capillary venous pressure. Using another method Garten (5) too found a drop in this pressure Muller and Rorvik (12, 13) found reduction of pulmonary capillary venous pressure in patients with coronary heart disease after nitroglycerine, at rest and especially during exercise.

Because the left ventricle in patients with mitral stenosis is protected by the stenosed mitral valve, this material is not very suitable in assessing the changes in the function of the left ventricle.

From this study it is difficult to find the primary effect of Synadrin on the circulation. Probably the cardiac output is reduced, either by reduction of the venous return to the right atrium or by reduction of the heart rate, possibly both occur simultaneously A reduced venous return may be caused by dilatation of the peripheral capacity vessels. Synadrin has sympatholytic effects (6, 10) and there is also a possibility that reduced contractility of the left ventricle is the reason for the reduced cardiac output. The oxygen consumption is almost unchanged during the estimation of cardiac output before and after injection of Synadrin, and this may suggest a "steady state"

The reduction of the cardiac output may cause a drop in the systemic blood pressure. The drop in the pulmonary capillary venous pressure may be explained as an effect of the changes in the cardiac output and heart rate.

A reduction of calculated pulmonary vascular resistance is found in the three patients with highest pressure in the pulmonary circulation. In the two patients (No. 2 and 6) with the lowest final pressure there is an increase in the resistance.

Rudolph and Auld (17) found a rapid decrease in pulmonary vascular resistance when the pulmonary venous pressure or the pulmonary artery pressure was increased. They suggest that the pulmonary vessels are passively distended by the pulmonary venous and pulmonary artery pressure, and this may explain the different reaction after Synadrin in the patients with high or low pressure in the pulmonary circulation.

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	a	b	a	b	a	b	a	b	a	b	a	b		b
1	18	16	9	10	165 /80	160 /80	4.9	4.3	70	68	70	63	147	112
2	7	7	6	3	130 /80	130 /85	4.9	4 6	89	72	55	64	16	70
3	33	20	16	13	130 /90	125 /85	6.0	6.0	115	86	52	70	227	93
4	18	19	–	–	115 /75	105 /60	–	–	50	54	–	–	–	–
5	81	81	–	–	110 /75	105 /75	–	–	99	94	–	–	–	–
6	13	11	10	7	115 /80	105 /75	8.9	8.6	80	79	111	109	27	37
7	23	13	16	12	120 /80	115 /80	3.6	3 4	90	75	40	45	156	24
Average	27.6	23.9	11 4	9.0	126.4/80	120.7/77 1	5 66	5.38	84 7	75.4	65.6	70.2	114.6	67.2
M. diff	–3 7		–2.4		–5 71/2.86		–0.28		–9.3		+ 4.6		–474	
P	0.01 < P < 0 05		Syst. 0.001 < P < 0.01				0.01 < P < 0.05							
t	1 78		2.75		Syst. 4.38		2.89		2.13		0.94		1.26	

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During the test there were no changes in the PQ or QRS intervals. Very large and repeated injections of Synadrin in animal experiments have resulted in lengthening of PQ and QRS (10) In human beings Brandschwede (2) too found practically no changes apart from an increase of the PQ interval of 0 03 seconds following injection of 20 mg in one patient.

Summary

Seven patients with mitral stenosis were given 5 mg Synadrin® (N [3 phenyl propyl (2')] 1 1 diphenyl propyl (3) amine) through a catheter in the pulmonary artery There was an insignificant reduction of the heart rate and pressure in the pulmonary artery The reduction of the systolic pressure in the systemic arteries was significant, but not the reduction of the diastolic and mean arterial pressures. There was a significant drop in the pulmonary capillary venous pressure and the cardiac output, but no significant change in the calculated pulmonary and peripheral vascular resistances.

It is possible that the reduction of the cardiac output is the primary effect of Synadrin This may be the result of a reduced venous return following dilatation of the peripheral capacity vessels, a drop in the heart rate or a reduction of the ventricular contractility all sympatholytic effects, or a combination of these factors. The drop in the systolic arterial pressure may be induced by the reduction of cardiac output, and the drop in the pulmonary capillary venous pressure by the changes in cardiac output and heart rate.

Acknowledgement

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Studies on the Administration of Streptokinase

A Method for Obtaining a Thrombolytic Effect Without Excessive Fibrinogenolysis

By

IVAR HELLE

with the technical assistance of I. THEODOR

Acute thromboembolic vascular disease is an important cause of illness and death in man. The development of clot-dissolving agents for the treatment of these disorders opens promising aspects in clinical medicine.

Since Tillett et al. (30) first demonstrated that infusion of streptokinase (SK) induced a transient fibrinolytic activity in man many workers (8, 11, 17, 23 a. o.) have published their results of such therapy. Much is known about the mechanism of the SK induced thrombolytic state (9, 27) but controversy still exists on such important problems as SK-dosage, duration of treatment, laboratory control during treatment and the kind of laboratory parameters to be used for evaluating the existence of a thrombolytic state in the patient (29).

This work deals with some practical questions concerning the SK-induced thrombolytic state and the mechanism of SK action in patients suffering from thromboembolic vascular diseases, especially acute myocardial infarction. A local application of SK in the coronary arteries (7) involves so many technical difficulties, that SK-treatment in such patients is carried out by inducing a systemic thrombolytic state.

Different SK doses and techniques to induce a thrombolytic state have been tried. The results suggest that a low initial dosage of SK will reduce the fibrinogenolysis and thereby probably the frequency of bleeding complications. When plasma plasminogen is near zero, it is found that large doses of SK can be given, resulting in a thrombolytic state, without further depression of plasma fibrinogen values and without bleeding complications.

Material

The clinical material consisted of 17 patients, aged from 41 to 80 years. The diagnoses were as follows: Acute myocardial infarction (13), cerebral thrombosis (2), venous thrombosis (1) and arterial thromboembolism (1).

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Table I Group I SK administration, plasma fibrinogen and bleeding complications. Infusion rate of SK: 3 TISD/hour for one hour

	TIS	Fibrinogen						Bleeding compl.
		Jacobsson (mg %)			Schneider (titer)			
		1)	2)	3)	1)	2)	3)	
M. N.	150	–	–	–	1/100	1/1	–	+
O. R.	50	383	–	37	1/400	1/25	1/10	+
T. N.	25	240	100	70	1/400	1/25	1/10	+
O. E. J	12.5	440	210	150	1/400	1/10	1/10	+
R. O. B.	12.5	480	310	240	1/800	1/50	1/50	–

TIS = Titrated initial SK sensitivity of whole blood (units/ml)
TISD = TIS × blood volume (calculated as 8 %/body weight).
1) = Fibrinogen before start of SK treatment.
2) = Fibrinogen immediately after SK treatment.
3) = Fibrinogen 3 hours after discontinuance of SK treatment.

tion. This state is termed "plasminogen exhaustion" in this paper. The test was performed 2 hours after the beginning of SK infusion and then every hour until the state of "plasminogen exhaustion" was reached.

P-P-determination. The method of Owren and Aas (26) was used.

SK administration. The dose of SK to be administered was dissolved in 300 to 500 ml of 5 % glucose and given by intravenous drip. The infusion needle was placed in a leg or cubital vein (on the side opposite the polyethylene catheter insertion). A new infusion bottle was prepared every four hours. Twenty mg prednisolone ("Glucortin" Cortec A/B, Copenhagen) was added to the first bottle. Fluid intake was restricted during infusion and not more than 1,000 ml glucose was given during the whole infusion period.

SK sensitivity of whole blood and calculation of SK dose. Prior to treatment the amount of SK necessary to produce total lysis of citrated, coagulated whole blood within 10 min. was determined. The titration was carried out as described by Nilsson and Olow (23). The smallest amount of SK giving a lysis time shorter than 10 min. was recorded. This SK dose is referred to as titrated initial sensitivity (TIS) of blood (units/ml).

For each patient, the SK dose to be used was calculated by multiplying TIS by the blood volume estimated as 8 % of the body weight. This dose is hereafter referred to as titrated initial sensitivity dose (TISD). All doses of SK administered are reported as multiples of TISD. Corrections for dilution by citrate, thrombin and SK solution have not been made.

Results

According to SK dosage, the material is divided into three groups. Group I and II both were given a total SK dose of 3 TISD. In group I this dose was given during one hour. In group II, the dose was given during two hours. In group III the dosage was different as described below.

As seen from table I and II the two first groups are comparable as to SK sensitivity. In group I thrombolytic activity was tested for and found in one patient only but was most probably present in all patients. In group II thrombolytic activity was recorded in one out of 3 patients tested. The plasma fibrinogen, however, was markedly reduced during treatment in both groups, especially in group I. The degree of

Anticoagulant Sodium citrate dihydrate 3 13 % was used for samples to be tested for fibrinolytic activity *ε-aminocaproic acid* (ε-ACA) A 10 % sterile solution in distilled water (A/B Kabi Stockholm) was used.

A stock solution containing 10 % ε ACA and 3 13 % sodium citrate dihydrate was used as anticoagulant for all samples collected for fibrinogen determination

Buffer A modified veronal buffer (pH 7 35 and ionic strength 0 15) (25) was used

Fibrinogen. Bovine fibrinogen (A/B Kabi, Stockholm) with a clottability of about 97 % was dissolved in saline to a one % solution and stored at — 20° C. After thawing, the fibrinogen was diluted in the veronal buffer to 0 12 % and used for the fibrin plates.

Sodium carbonate anhydrous, guaranteed reagent (E. Merck, Darmstadt) A stock solution of 0 1 in distilled water was used

Streptokinase (Kabikinase" A/B Kabi Stockholm) containing 66 Christensen units per μg protein was used. 10 000 units were dissolved in 5 ml 0 9 saline for the SK sensitivity tests. Fresh solution was prepared each day Vials containing 250 000 units were used for infusion and prepared as described below

Thrombin. Bovine thrombin "Topostasin" (Hoffman-La Roche, Basel) was dissolved in 0 9 % saline in plastic tubes to a final concentration of 30 N I H. units/ml and stored at — 20° C. After thawing, the stock solution was further diluted to the desired strength and used immediately

Methods

Collection of blood. Blood samples were drawn through a polyethylene catheter (Braunule, B. Braun, Melsungen) from an arm vein. Blood samples were taken from each patient before, during and at various intervals after infusion. The blood was collected in plastic tubes. Nine parts of blood and one part of anticoagulant were thoroughly mixed and placed on melting ice. Citrated whole blood was immediately examined for SK sensitivity The rest of the blood was centrifuged at + 4 C for 30 min. at 2,500 r p.m. The platelet poor plasma was immediately pipetted off and, if not examined at once, stored at — 20° C.

Platelet-poor red cell suspension was prepared as described by Blix (5)

Fibrinogen determinations. The citrate-ε ACA mixture was used as anticoagulant and to avoid further fibrinogenolysis. Two methods have been used throughout a) determined as fibrin after coagulation with thrombin by the method of Jacobsson (15) with the modifications of Blombäck and Blombäck (6) and Godal (12) b) determined by the dilution method of Schneider (28) 0.2 ml of thrombin (30 N I.H units/ml) was added to each fibrinogen dilution and the results read after 30 min.

Fibrinolytic activity a) Unheated and heated fibrin plates. The methods of Astrup and Mullertz (4) and Lassen (21) were used. The heated plates were kept at 85 C for half an hour The tests were incubated at 37° C for 20 hours. The product of two perpendicular diameters of the lysed area was recorded as the measure of fibrinolytic activity b) Thrombolytic activity Thrombolytic activity is in this study used to describe the ability of patients plasma to lyse a preformed clot. For this purpose we have found the method introduced by Blix (5) convenient. The plasma clots were made from 0.4 ml platelet-poor red cell suspension from the patient + 0.7 ml citrated plasma (obtained from the patient prior to the SK treatment) + 0 1 ml thrombin (20 N I H. units/ml) The clots were allowed to stand at room temperature for 20 min. before use. Undiluted citrated plasma to be tested for thrombolytic activity was used and the clot was suspended in 2 8 ml of this plasma. Plastic tubes containing clot and plasma were placed on a rotating axis (10 r p.m.) in a water bath at 37° C. 0.2 ml plasma were removed after 1 and 2 hours incubation and diluted with 2 8 ml 0 1 % sodium carbonat for hemoglobin determination. The transmission was read in a Beckman B spectrophotometer at 540 mμ against plasma sodium carbonate as a blank. A reference curve was made for each patient and the per cent clot lysis was calculated as described in the original method. If 25 % or more of the clot was lysed during an incubation period of hours, a thrombolytic effect was recorded

Plasminogen exhaustion" A lysis time of more than 20 min. for citrated whole blood after addition of thrombin and the five-fold TIS-SK-dose (see below) was considered to indicate negligible plasminogen concentra-

Table IV Group III Plasma fibrinogen and bleeding complications

	Fibrinogen						Bleeding compl.
	Jacobsson (mg %)			Schneider (titre)			
	1)	2)	3)	1)	2)	3)	
H. J	440	220	130	1/1,600	1/50	1/50	–
M. P	480	280	240	1/400	1/50	1/25	–
H. P	730	560	420	1/800	1/400	1/100	–
B. L.	430	210	190	1/200	1/25	1/25	–
C. M.	390	240	190	1/400	1/200	1/100	–
M. A.	400	270	220	1/400	1/25	1/25	–
E. T. M.	410	270	240	1/400	1/50	1/50	–
B. T	430	270	210	1/400	1/23	1/10	–

1) – Before SK treatment.
2) – Immediately after "plasminogen exhaustion (1st period).
3) – Immediately after the 2nd period ("thrombolytic period").

Table V Group III Fibrinolytic activity

	The 1st ("fibrinogenolytic") period			The 2nd ("thrombolytic") period		
	1)	2)	3)	1)	2)	3)
H. J	110	72	+	500	0	+
M. P	504	100	–	1,292	0	+
H P	–	–	+	875	0	+
B. L.	440	81	–	1,326	0	+
C. M.	360	64	+	900	0	+
M. A.	462	49	–	650	0	–
E. T. M.	506	64	–	676	0	+
B. T	309	42	+	990	0	+

1) Fibrinolytic activity (unheated fibrin plates-mm, lysed area)
2) – Fibrinolytic activity (heated fibrin plates-mm, lysed area).
3) – Thrombolytic activity (+ more than 25 % of the clot lysed during 2 hours incubation)

fibrinogen depression seemed to be more pronounced when measured by the method of Schneider (28) than by the method of Jacobsson (15) In four out of five patients in group I the fibrinogen titre was lower than 1/25 as measured by the method of Schneider Two of these patients had moderate oozing bleeding from the gingiva and two revealed small echymotic areas around the venipuncture sites. In group II where the SK doses were infused more slowly a slighter degree of fibrinogen depression was noted and the patients showed no tendency to bleed.

A typical example from group I is shown in more detail (fig 1) The decreasing fibrinogen values correspond well to a persisting plasmin activity as measured on heated fibrin plates.

Group III (8 patients) Approximately 0.3 TISD/hour was given until pla

Table II Group II SK administration, plasma fibrinogen and bleeding complications. Infusion of SK 1.5 TISD/hour for 2 hours

	TIS	Fibrinogen						Bleeding compl.
		Jacobsson (mg %)			Schneider (titer)			
		1)	2)	3)	1)	2)	3)	
K. A. S.	50	280	200	190	1/200	1/100	1/50	–
W. M.	25	380	280	280	1/200	1/100	1/100	–
B. S.	25	300	210	180	1/200	1/50	1/50	–
H. J.	12.5	370	280	240	1/400	1/200	1/100	–

TIS = Titrated initial SK sensitivity of whole blood (units/ml)
TISD = TIS × blood volume (calculated as 8 %/body weight)
1) = Fibrinogen before start of SK treatment
2) = Fibrinogen immediately after SK treatment
3) = Fibrinogen 2 hours after discontinuance of SK treatment

Table III Group III SK administration

	Weight (kg)	TIS	TISD	The 1st ("fibrinogenolytic") period		The 2nd ("thrombolytic") period		
				1)	2)	1)	2)	3)
H. J.	70	25	140 000	1.0 0.3	1.6 4.0	1.8	1.5	0.73
M. P.	50	25	100 000	0.6 1.0	3.3 2.5	2.6	3.75	1.43
H. P.	50	25	100 000	0.2 0.8	2.0 2.0	5.4 0.6	0.5 2.0	0.625
B. L.	79	12.5	81,500	0.3 1.0	1.5 2.5	See fig. 2		1.0
C. M.	75	25	150,000	0.6	3.5	5.2	0.33	0.57
M. A.	70	25	140 000	0.4	8.0	1.8	2.0	0.96
E. T. M.	90	25	180,000	0.56	2.0	1.6	2.5	1.0
E. T.	85	50	340 000	0.3	3.0	1.5 0.7	1.1 2.5	1.5

TIS = Titrated initial sensitivity to SK of whole blood (units/ml)
TISD = TIS × blood volume (calculated as 8 %/body weight)
1) = SK infusion rate (TISD/hour)
2) = SK infusion time (hours)
3) = Total dose of SK infused (units × 10^6)

Table IV Group III Plasma fibrinogen and bleeding complications

	Fibrinogen						Bleeding compl.
	Jacobsson (mg %)			Schneider (titer)			
	1)	2)	3)	1)	2)	3)	
H. J.	440	220	150	1/1,600	1/50	1/50	—
M. P.	480	280	240	1/400	1/50	1/25	—
H. P.	730	360	420	1/800	1/400	1/100	—
B. L.	430	210	190	1/200	1/25	1/25	—
C. M.	390	240	190	1/400	1/200	1/100	—
M. A.	400	270	220	1/400	1/25	1/25	—
E. T. M.	410	270	240	1/400	1/50	1/50	—
B. T.	430	270	210	1/400	1/25	1/10	—

1) = Before SK treatment.
2) = Immediately after "plasminogen exhaustion" (1st period).
3) = Immediately after the 2nd period ("thrombolytic period").

Table V Group III Fibrinolytic activity

	The 1st ("fibrinogenolytic") period			The 2nd ("thrombolytic") period		
	1)	2)	3)	1)	2)	3)
H. J.	110	72	+	500	0	+
M. P.	584	100	—	1,292	0	+
H. P.	—	—	+	875	0	+
B. L.	440	81	—	1,326	0	+
C. M.	360	64	+	900	0	+
M. A.	463	49	—	650	0	—
E. T. M.	506	64	—	676	0	+
B. T.	309	42	+	990	0	+

1) Fibrinolytic activity (unheated fibrin plates-mm² lysed area).
2) Fibrinolytic activity (heated fibrin plates-mm² lysed area).
3) = Thrombolytic activity (+ = more than 25 % of the clot lysed during 2 hours incubation).

fibrinogen depression seemed to be more pronounced when measured by the method of Schneider (28) than by the method of Jacobsson (15). In four out of five patients in group I the fibrinogen titre was lower than 1/25 as measured by the method of Schneider. Two of these patients had moderate oozing bleeding from the gingiva and two revealed small echymotic areas around the venipuncture sites. In group II where the SK doses were infused more slowly a slighter degree of fibrinogen depression was noted, and the patients showed no tendency to bleed.

A typical example from group I is shown in more detail (fig. 1). The decreasing fibrinogen values correspond well to a persisting plasmin activity as measured on heated fibrin plates.

Group III (8 patients). Approximately 0.5 TISD/hour was given until plas-

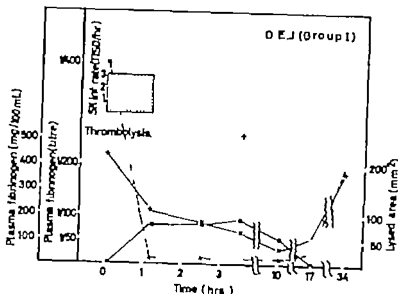


Fig 1 Fibrinogen, proteolytic activity (heated fibrin plates) and thrombolytic activity in patient O.E.J (group I) during SK treatment.

×——× Fibrinogen (mg %)
×– – –× Fibrinogen titer
●——● Fibrinolytic activity (heated fibrin plates) mm, lysed area.

minogen exhaustion" occurred followed by very large doses of SK (from 1 5 to 5.2 TISD/hour) The results are given in table III IV and V

No evident correlation between the SK dosage and the degree of plasma fibrinogen depression was observed. In all cases, fibrinogen decreased markedly until "plasminogen exhaustion but in no instances was a fibrinogen titre lower than 1/25 observed, and further no bleeding complications occurred In four cases, eventual thrombolytic activity was searched for with negative result. "Plasminogen exhaustion was achieved in all patients within 2—8 hours.

The SK doses given during the second period varied from 1 5 to 5 4 TISD/hour The highest dosage, 1 7 TISD (250 000 units) was given during 20 minutes, without bleeding complications. A very high fibrinolytic activity (unheated fibrin plates) and a marked thrombolytic effect were found in all patients (table V) whereas no plasmin effect (heated plates) could be demonstrated During this period the fibrinogen titre remained nearly constant.

A typical example from group III is given in fig 2. The marked initial fibrinogenolysis, lasting until plasminogen exhaustion" and followed by nearly constant fibrinogen values in spite of a large increase in SK dose, is clearly demonstrated.

To all the patients in group III we have given the usual dose of the oral anticoagulant phenylindandione (commonly 160 mg) immediately prior to the SK infusion. In most cases, therapeutic P P values were obtained in 24 hours. Phenylindandione was given during hospitalization or longer When SK infusion was discontinued 7,500 units of heparin were given intravenously and repeated 4 hours later In two cases heparin was given during SK treatment without bleeding complications. One patient on permanent anticoagulant treatment revealed no further depression of the P P value during SK therapy

In 14 patients, 20 mg prednisolone was added to the first infusion bottle and four hours later 10 mg prednisolone was given per os In 3 cases steroids were not given and all these had a short febrile reaction.

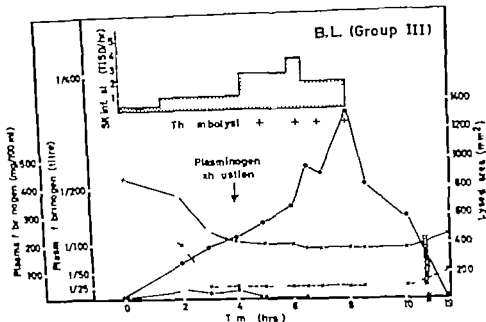


Fig. 2. Fibrinogen, fibrinolytic and thrombolytic activity in patient B.L. (group III) during SK treatment.

—— Fibrinogen (mg %)
× × Fibrinogen titer
Fibrinolytic activity (unheated fibrin plates) mm² lysed area.
●——● Fibrinolytic activity (heated fibrin plates) mm² lysed area.

Comments

Controversy still exists regarding the mechanism of SK-induced thrombolysis (1 3) Preliminary in vitro studies in this laboratory confirm the results of Alkjaersig et al. who found that the thrombolytic effect of SK does was due to activation of clot plasminogen by the SK activator complex in the surrounding plasma (14)

The effect of SK is reduced by the action of specific SK inhibitors (16) and unspecific plasmin inhibitors (24) Since such inhibitors can be removed by fractionation of plasma (euglobulin precipitation) or their effect abolished by simple dilution, in vitro estimation of the thrombolytic effect of SK must be carried out under conditions where both activators and inhibitors in plasma are fully represented. In this study we have therefore used a special clot system. The undiluted citrated plasma from treated patients acts on a clot formed from the patient's plasma obtained prior to SK treatment (extra clot lysis system) The test system used in this work is probably less sensitive than the euglobulin lysis test (intra clot lysis system) and the fibrin plate method. Probably the extra clot lysis system more closely simulates the intended in vivo effect than the intra clot system. If only very high values of activity are taken to reflect a thrombolytic state, the fibrin method may also be used

Therefore, in order to obtain a thrombolytic effect, relatively high concentrations of SK activator complex in plasma are necessary As shown by Alkjaersig et

al. (2) and Fletcher et al (10) the SK induced plasminogen activation involves breakdown of plasma fibrinogen (fibrinogenolysis) The split products inhibit the last stage of coagulation and are partly incorporated into the clot. It is reasonable to believe that such defective polymerization is partly responsible for the bleeding complications so frequently observed during treatment with large amounts of SK. Since the rate of fibrinogenolysis is roughly proportional to the fibrinolytic activity present, and since fibrinogen is at least as sensitive to SK as fibrin (13) fibrinogenolysis is probably inevitable. An initial amount of SK sufficient to induce a thrombolytic state, will as shown in our group I result in a massive fibrinogenolysis and bleeding complications. According to data presented in group III it is possible to reduce the degree of fibrinogenolysis and probably also the frequency of bleeding complications by using a relatively low initial SK dosage. After various periods of time plasma becomes far less sensitive to fibrinogenolysis by SK probably due to plasminogen depletion (9) Thereafter very large amounts of SK can be given without further reduction of plasma fibrinogen and without bleeding complications, whereas the thrombolytic effect of plasma samples tested on preformed plasma clots is pronounced. It must therefore be concluded that a pronounced thrombolytic effect can most likely be obtained by using the method of administration applied in group III In fact, as to fibrinogenolysis there seems to be no upper limit for SK doses which can be infused *after* "plasminogen exhaustion This observation is of great practical importance since it might be possible to reduce the duration of treatment with an even stronger thrombolytic effect.

The SK treatment carried out as in group III can be divided into a) a fibrinogenolytic period and following plasminogen exhaustion" b) a thrombolytic (or therapeutic) period.

The first period is characterized by plasmin formation leading to fibrinogenolysis but little or no thrombolytic effect as tested on preformed plasma clots. It is a disadvantage that the fibrinogenolytic period is inactive as regards thrombolysis, but further progression of a thromboembolic process should be prevented. In other words, this period is *thromboprevention* The SK dosage given by us during this period is still as intense as the SK dosage used by other workers to obtain a therapeutic effect (8 9 22 a. o)

The second period is characterized by high SK activator concentration in plasma, giving a strong thrombolytic effect but no evident plasmin effect, and moderate fibrinogenolysis.

It may be argued that the administration technique used by us involves the risk of formation of SK resistant thrombi after plasminogen exhaustion (19) However since 'plasminogen exhaustion occurs during prolonged infusion of SK doses below the thrombolytic range as judged by the extra clot lysis system, this risk is probably inevitable.

In order to minimize this risk, patients in group III received heparin immediately after discontinuance of SK treatment. Perhaps heparin should also be used during the thrombolytic period particularly because heparin in small doses is said to enhance the fibrinolytic effect in vivo (20)

It should be borne in mind that Kellner and Robertson (18) have noted lesions resembling Aschoff bodies in the myocardium of rabbits given large

amounts of SK. No examinations as to this problem have been carried out in this study as all our patients survived.

Conclusions and summary

SK infusions have been administered to 17 patients. Different SK administration techniques have been tried.

It is shown that the initial infusion period is characterized by a plasmin effect, leading to marked fibrinogenolysis, roughly proportional to the SK doses used. Fibrinogenolysis is probably inevitable when doses sufficient to induce a thrombolytic state are used. The degree of fibrinogenolysis can be limited by using a relatively low initial dosage of SK.

For estimation of fibrinogenolysis, the dilution method of Schneider was found to be convenient. In 4 out of 5 patients with a fibrinogen titer below 1/25 a bleeding tendency was noticed.

A depletion of plasma plasminogen has been achieved in 8 patients. After plasminogen exhaustion very large SK doses were given, inducing a strong thrombolytic effect as tested on preformed plasma clots, but without a further decrease in fibrinogen values as estimated by the method of Schneider and without bleeding complications.

The SK treatment has been combined with anticoagulants, without bleeding complications.

Acknowledgement

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Effect of Intravenous Injection of Heparin in Varying and Repeated Doses on the Coagulation Time

An Experimental Study on Dogs

By

Per Olsson

Experiments on human subjects concerning the variation in plasma heparin content, as well as in the coagulation time after intravenous injection of heparin, have been reported in an earlier paper from this laboratory (2). At the same heparin concentration in the blood, considerable differences were found between the coagulation time in different individuals. However in these experiments, the heparin doses ranged from 200 to 600 I.U./kg body weight and the results indicated that the heparin activity in the plasma required for a certain prolongation of the coagulation time is higher after a large dose than after a small one. For evaluation of these findings, it seemed important to investigate the anticoagulant potency of different heparin doses.

An account is given in the present paper of experiments on dogs, in which the coagulation time was repeatedly determined after intravenous injection of different amounts of heparin. The effect of a repeated injection of heparin was also investigated.

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Material and methods

The material consisted of 8 mongrel dogs, ranging in weight from 15 to 22 kg. They were anaesthetized by intraperitoneal injection of 10 mg/kg body weight of Nembutal® (Abbott) and intubated tracheally. If necessary additional Nembutal was given intravenously. For blood sampling, a polyethylene catheter (PE 190) was introduced into the inferior vena cava via a peripheral vein in one hind leg.

Heparin administration

Commercial heparin (Vitrum Comp Stockholm) in 5 % solution, containing about 130 I.U./mg was administered by venous puncture of a foreleg. Two dogs were given 200 I.U./kg body weight, 2 dogs 400 I.U./kg and 2 dogs 800 I.U./kg. All these dogs received repeated dose of the same quantity of heparin 6 hours later. In the remaining 2 dogs the first heparin injection was omitted, but the same number of samples was taken as in the other dogs. A single dose of 400 and 800 I.U./kg body weight, respectively was then given at the time for scheduled second dose of heparin.

Heparin activity in plasma

This was measured before the heparin injection and every 20 minutes after it, until the coagulation time was normal. Each blood sample (4.5 ml) was withdrawn from the vena

Table I Changes in plasma heparin activity (IU/ml plasma) after injection of heparin. Second injection given 6 hours after first one

Time (min) after heparin inj.	800 IU/kg BW				400 IU/kg BW				200 IU/kg BW				800 IU/kg BW		400 IU/kg BW	
	Dog 785		Dog 779		Dog 783		Dog 793		Dog 788		Dog 789		Dog 823		Dog 710	
	Inj. I	Inj. II	Inj. I	Inj. II	Inj. I	Inj. II	Inj. I	Inj. II	Inj. I	Inj. II	Inj. I	Inj. II	No hep.	Hep. inj.	No hep.	Hep. inj.
0	0 1	0 1	0.3	0.3	0.2	0.3	0.1	0.2	0.2	0.2	0.1	0.3	0.3	—	0.3	—
20	14 6	14 0	9 0	9 4	5.6	5.7	5 4	6.0	2.2	2.2	2.4	2.2	0.3	10.1	0.1	5.0
40	10.2	10 7	7 1	7 4	4.8	4 7	3.8	4 1	1 6	1.6	1.8	1.8	0.1	9.3	0.2	3.9
60	9 6	8.4	5 1	5 4	3.5	3.6	2.6	3.8	1.3	1 1	1 4	1 4	0.2	6.8	0.2	3.2
80	6.6	6.8	4.4	4.5	2.6	3.2	1.9	2.2	0.8	0.7	1.1	1 0	0.3	6.4	0.0	2.8
100	5.9	5.6	3.8	3.2	2.4	2.3	1 4	1.3	0.7	0.6	0.8	0.8	0.0	5.4	0.0	2.3
120	5.0	5.0	3.0	2.7	1.9	1 7	1 1	1 1	—	—	—	—	0.3	4 7	0.3	2.0
140	4 0	4 0	—	—	—	—	—	—	—	—	—	—	—	4.1	—	1 7

Table II

Dog	Dose (IU/kg BW)	Inj.	Theoretical initial hep. activity (IU/ml plasma)	Time (min) for 50% decrease in hep. activity
785	800	I	16.7	67
		II	16 1	68
779	800	I	10.7	65
		II	12.1	54
783	400	I	7 0	62
		II	7.5	59
793	400	I	7.2	43
		II	8.3	41
788	200	I	2.9	47
		II	3.1	41
789	200	I	3.1	51
		II	2.9	53
823	800	—	11.8	90
710	400	—	5 6	79

Second heparin injection given 6 hours after first one.
Single heparin injection given at time for scheduled second injection.

cava into a syringe containing 0.5 ml of 3.8 % sodium citrate the heparin activity of the plasma was then determined by the method of Blombäck et al. (1)

In order to measure the relative elimination rate of the injected heparin the straight line which had the best coincidence with the semilogarithmic elimination curve was calculated in each experiment. The time for 50% decrease in heparin activity was then determined from the b value (angle coefficient) obtained with the formula $T/2 = \frac{\log 2}{b}$

Coagulation time

This was measured before the heparin injection, 10 minutes after it, and then every 5 minutes during the early part of the experiment, and about every 10 minutes during the later part. The samples for these determinations were withdrawn from the caval catheter into 5 ml syringes, after discarding the first 2 ml. One half to one ml of blood was carefully transferred into a glass tube (8 × 55 mm) which was sealed and placed in a water bath at 30° C. The tubes were gently tilted and rotated every 2 minutes, and the blood film transilluminated with daylight. The end-point of coagulation was taken as the first appearance of fibrin threads, and the time was noted. If no fibrin was formed in 6 hours, the coagulation time was regarded as immeasurably prolonged.

Table III. Changes in coagulation time (min) after injection of heparin. Second injection given 6 hours after first one

Time (min) after heparin inj.	800 IU/kg BW				400 IU/kg BW				200 IU/kg BW				800 IU/kg BW		400 IU/kg BW	
	Dog 783		Dog 779		Dog 783		Dog 793		Dog 786		Dog 789		Dog 823		Dog 710	
	Inj. I	Inj. II	Inj. I	Inj. II	Inj. I	Inj. II	Inj. I	Inj. II	Inj. I	Inj. II	Inj. I	Inj. II	No hep.	Hep. inj.	No hep.	Hep. inj.
0	3	4	4	3	3	3	4	5	4	4	3	4	5	—	4	—
10	∞	20	∞	28	48	12	∞	30	17	12	43	14	—	∞	—	∞
15	∞	16	∞	16	30	7	78	—	11	5	29	8	—	∞	—	56
20	∞	19	74	11	16	8	38	12	7	9	19	7	—	∞	—	37
25	∞	24	45	7	20	6	29	15	6	6	16	5	3	∞	3	22
30	80	19	34	9	12	6	25	12	8	6	10	6	—	82	—	20
35	52	23	29	9	11	5	16	11	6	4	7	—	—	53	—	14
40	26	12	18	11	8	5	15	12	—	4	4	4	3	48	5	11
45	23	9	18	7	9	—	14	9	—	4	4	4	—	30	—	8
50	21	10	17	—	6	5	9	10	5	—	5	4	—	25	—	10
55	22	12	13	5	11	—	12	12	5	4	5	—	3	—	4	9
60	21	11	16	6	10	5	8	11	4	—	4	4	—	26	—	—
65	—	12	13	7	7	—	11	8	6	4	4	—	—	—	—	10
70	20	12	10	—	6	—	—	6	4	—	4	4	5	16	3	7
75	13	8	11	7	5	—	7	3	—	—	4	—	—	20	—	8
80	13	10	12	5	6	5	8	5	4	4	4	4	—	—	—	6
85	18	—	7	—	6	—	7	7	4	—	4	4	4	12	4	5
90	18	8	8	5	—	—	4	—	—	—	—	—	—	—	—	6
95	15	9	3	—	5	—	4	4	—	—	—	—	—	15	—	—
100	16	6	—	4	6	—	4	4	4	—	—	—	—	—	—	—
105	13	—	8	6	5	—	4	—	—	—	—	—	4	10	4	5
110	8	9	7	—	—	—	—	—	—	—	—	—	—	7	—	—
115	11	7	3	5	5	—	4	4	—	—	—	—	—	—	—	4
120	9	5	4	—	4	—	—	—	—	—	—	—	—	—	—	—
125	8	4	—	—	—	—	—	—	—	—	—	—	—	8	—	4
130	4	5	—	—	—	—	—	—	—	—	—	—	4	—	5	—
135	—	—	—	—	—	—	—	—	—	—	—	—	—	4	—	—
140	6	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—
145	—	—	—	—	—	—	—	—	—	—	—	—	—	6	—	—
150	5		—	—	—	—	—	—	—	—	—	—	—	—	—	—
155	—	—	—	—	—	—	—	—	—	—	—	—	—	4	—	—

Results

Heparin activity in plasma

The variations in plasma heparin activity in the different experiments are seen in table I. Before injection of heparin the value ranged from 0.1 t 0.3 IU ml of plasma, nd t the time of the second injection t had returned essentially to the initial level. The concentration fell in a single exponential regression, nd the time for 50 % decrease obtained from the slope of the regression line ranged from 41 to 90 minutes in the

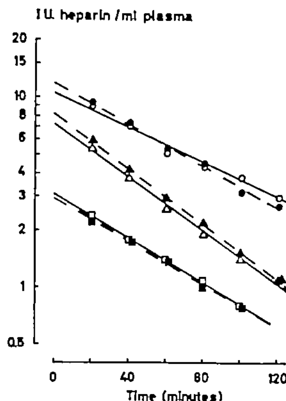


Fig 1 Elimination of intravenously injected heparin from plasma.
○—○ and ●—● 800 I U /kg BW (dog 779)
△—△ and ▲—▲ 400 I.U /kg BW (dog 793)
□—□ and ■—■ 200 I.U /kg BW (dog 789)
Open dots: 1st injection.
Filled dots: 2nd injection.

total material No significant difference was present between the values after the first and second injection, respectively in the individual animals (table II) Typical elimination curves are shown in fig 1

To obtain an expression of the size of the heparin dose in relation to the plasma volume, the theoretical plasma heparin activity directly after heparin injection was extrapolated from the regression line in each experiment. The values are seen in table II In this respect as well no significant difference was present between the first and second injection in any of the dogs

Coagulation time

The changes in coagulation time in the different experiments are listed in table III Before injection of heparin, the value ranged from 3 to 5 minutes, and no difference was found between the values before the first and second injection respectively Shortly after the first injection of heparin, a marked prolongation in coagulation time was noted in most cases, with a subsequent rapid decrease. In both dogs given 800 I U /kg as well as in one given 400 I.U /kg the prolongation was, in fact, immeasurable for a varying period after injection

In every case, the second injection of heparin had less effect. Immeasurable prolongation of the coagulation time did not occur in any of the dogs, and a normal value was invariably reached in a shorter time than after the first injection. However in the two dogs given a single heparin injection at the time for a scheduled second injection, the changes in coagulation time followed the same pattern as after a first injection.

Correlation between coagulation time and plasma heparin activity

The coagulation time obtained in each determination was correlated to the corresponding plasma heparin activity which was read off on the regression line for heparin elimination Typical curves are shown in fig 2. The decrease in coagulation time during elimination of a heparin dose was greater with each unit fall in plasma heparin at the beginning of the period than later After a first dose, a linear relationship between plasma heparin activity and the logarithm of coagulation time was, in fact, present in most cases. After the second injection, the

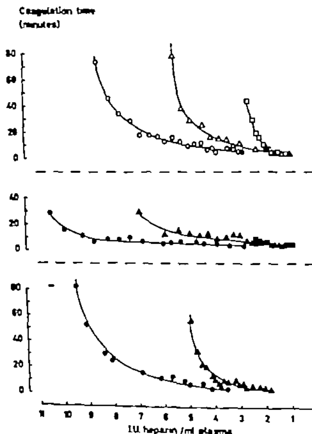


Fig. 2. Correlation between coagulation time and heparin activity in plasma.
○—○ and ●—● 800 I.U./kg BW (dog 779)
△—△ and ▲—▲ 400 I.U./kg BW (dog 793)
□—□ and ■—■ 200 I.U./kg BW (dog 789)
Open dots 1st injection.
Filled dots 2nd injection.
⊗—⊗ 800 I.U./kg BW (dog 823)
⚠—⚠ 400 I.U./kg BW (dog 710).
Single doses given at the time for scheduled 2nd dose.

anticoagulant potency of the injected heparin was considerably reduced as compared with that after the first injection thus, at corresponding plasma levels, the coagulation time was always shorter. Moreover there was a definite tendency to normal coagulation time being reached at a higher heparin activity after the second injection than after the first one.

In the dogs given single heparin injection, the correlation curves between coagulation time and plasma heparin activity had the same appearance as those after a first injection. Consequently they were regarded as reflecting conditions equivalent to those after a first injection.

Relationship between heparin dose and prolongation of coagulation time

In the dogs given 400 I.U./kg body weight, the coagulation time after the first dose was markedly and even immeasurably prolonged at plasma heparin levels which, in dogs given 800 I.U./kg induces only a moderate prolongation. Similarly the coagulation time in the dogs given 200 I.U./kg was greatly prolonged at heparin levels where, in the 400 and 800 unit groups, it had returned to the normal value or close to it (fig. 2). Thus, the anticoagulant effect of a certain heparin activity in plasma decreased when the dose was increased. This was particularly evident after the first injec

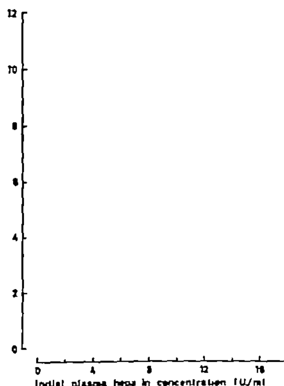


Fig 3. Correlation between initial plasma heparin activity following a first heparin injection and plasma heparin activity required for a coagulation time of 45 min.

tion For example, after a first dose which gave an initial estimated heparin activity of 7 0 I U /ml of plasma a coagulation time of 45 minutes required an activity of 6.2 I U /ml After a heparin dose giving an initial activity of 16 7 I U /ml the same coagulation time required a heparin activity of 10 5 I U /ml Fig 3 shows the correlation between the heparin activity necessary for 45 minutes coagulation time after a first injection (as well as after a single injection at the time for a sched uled second dose) and the initial activity produced by the dose in question. It is seen that the heparin activity required increased by about 0 6 I U /ml of plasma with each unit increase in initial activity

Discussion

The true course of heparin elimination in the dog has been found to be curved on the semilogarithmic scale (8) The deviation from the straight line was, however shown to be so slight that — from the practical point of view — it can be considered correct to read off the heparin activity at different times on a straight regression line. Considerable differences were present between individual animals with respect to the plasma heparin activity at a certain time after injection of a certain dose. Consequently when studying the anticoagulant effect of heparin it seems more adequate to relate the prolongation of the coagulation time to the corresponding plasma heparin activity rather than to the time after injection of the heparin The coagulation time measured by the appearance of fibrin threads — as in the present study — is shorter than that in similar investigations in which it has been determined by the formation of a clot.

Jaques and Ricker (5) found a linear relationship between heparin activity and the logarithm of coagulation time, when heparin was added to canine blood *in vitro* The present results indicate that such a correlation may also exist *in vivo* after a single intravenous dose of heparin. It is, however evident that the prolongation of coagulation time following such an injection is not dependent only on the heparin activity in plasma. Relatively seen the anticoagulant potency of heparin was found to decrease gradually with an increase in the dose especially when the coagulation times were long and was considerably diminished on repeated injection. The decrease in anticoagulant effect also seemed to be more marked after a large dose than after a smaller one.

The observation of a relative decrease in effect with increasing doses is not necessarily associated with the method used for determination of the coagulation time. Thus, Reinert and Winterstein (9) found, in measurements of the coagulation time in a mixture of saline and blood in rabbits, that the duration of heparin effect did not increase parallel with an increase in the dose. Eiber and Danishefsky (5) who followed the elimination of injected radioactive heparin in dogs, and measured the coagulation time according to Lee and White (6) also observed that an increase in the dose failed t produce a corresponding increase in anticoagulant effect. In the aforementioned studies, the coagulation time was not correlated to the heparin concentration in the blood. It is, however apparent from the data given by Eiber and Danishefsky that such correlation curves would be similar to those obtained in the present investigation. These and the present observations can largely explain the wide range in coagulation time in different animals, with the same heparin concentration in the blood, as reported by Monkhouse et al. (7) in experiments on dogs.

In the present experiments, the decreased anticoagulant effect of a repeated heparin dose was not due to anaesthesia or withdrawal of blood samples during elimination of the first dose. This is evident from the fact, that in animals given a single injection at the time for a scheduled second injection, the correlation curves between coagulation time and plasma heparin level resembled those after an initial dose. Administration of heparin thus seems to produce secondary reactions which inhibit the anticoagulant effect as well as the duration of the effect. Reinert and Winterstein (9) suggested an increase in thrombin activity following administration of heparin. However Howell and Holt (4) in *in vitro* experiments, showed that heparin exerts its anticoagulant effect by activating an antithrombin, i.e. that subsequently named the heparin co-factor Volkert (10) and Blombäck et al. (1) showed, also in *in vitro* experiments, that the co-factor content in plasma could be a limiting factor for the anticoagulating potency of heparin. The present results variations in effect on the coagulation time following intravenous administration of heparin under different conditions may therefore, depend on variations *in vivo* in the blood content of the heparin co-factor Studies of this problem are now being made, and will be reported.

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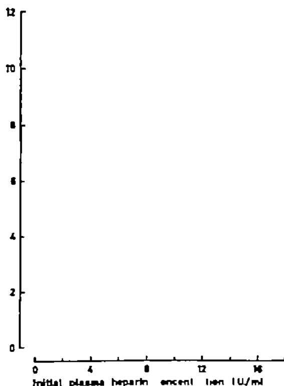


Fig 3 Correlation between initial plasma heparin activity following a first heparin injection and plasma heparin activity required for a coagulation time of 45 min.

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From the Central Laboratory Kommunehospitalet, Århus, Denmark

Studies on the Osmotic Fragility of Normal Human Erythrocytes

III. The Effect of Incubation on the Fragility of Erythrocytes

By

Esper Mortensen

The diagnostic significance of changes in red blood cell fragility after incubation for 24 hours at 37° C seems well established (3). Mild cases of hereditary spherocytosis can be differentiated from normal subjects by their abnormally increased osmotic fragility after incubation (3, 21, 26, 27). The distinction between the various types of congenital nonspherocytic haemolytic anaemia can be accomplished by the use of the osmotic fragility test after incubation in connection with determination of the degree of autohaemolysis after incubation (3).

The fragility tests applied differ with respect to important details of technique, and the normal values given are not directly comparable. Usually the normal ranges of osmotic fragility after incubation are considerably wider than the ranges of osmotic fragility without incubation (3). This implies, that even with normal blood uncontrolled variable factors may be operating during the incubation test resulting in a widening of the normal ranges. The following investigations were carried out in order to devise a set of normal values with the method previously described (16) and to evaluate the importance of some of the factors, which might influence the changes of the blood during incubation viz. the temperature of incubation, the time, the pH of the blood at the beginning of incubation, the packed cell volume (PCV) and the concentration of Ca^{++} and glucose during the period of incubation.

Material and methods

Blood from healthy volunteers was used throughout the investigations. The following routine tests were performed by the methods previously described (16): determination of haemoglobin, erythrocyte count, leukocyte count, reticulocyte count, PCV, ESR, serum bilirubin, serum creatinine, plasma iron and total iron binding capacity of plasma.

All values obtained were within the normal limits of the methods applied.

The normal values of osmotic fragility after incubation were based upon observations made on blood from 22 adult, healthy persons. The heparinized blood was subjected to the

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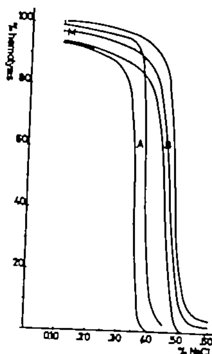


Fig 1 The normal values of the osmotic fragility test A) before and B) after incubation for 24 hours at 37° C. A represents the 95 % range. B represents the 95 range and the mean (M)

osmotic fragility test developed by the author (16) immediately after the venipuncture and after incubation at 37 C for 24 hours. The haemolytic solutions applied after incubation were equivalent to the following NaCl-solutions (concentrations in v/v) 0 10—0.26 — 0.32 — 0.38 — 0 42 — 0 44 — 0 46 — 0 48 — 0.50 — 0 52 — 0.54 — 0.56 — 0 60 %. In the fragility test without incubation the concentrations previously described were used.

All determinations were made in duplicate.

Variation of the temperature Incubation of blood samples from the same person was performed at the same time at 3 thermostatically controlled levels viz. 5—25—37° C.

Variation of the period of incubation. Blood from one person was examined after incubation for 0—12—24—36—48—60—72 hours. Samples from six persons were examined after 0—8—16—24 hours of incubation.

Variation of the pH of the blood was accomplished by adding calculated amounts of Na_2CO_3 and lactic acid to blood samples from one person producing values of standard bicarbonate within the clinically occurring levels.

Table I Values obtained in 22 healthy adult persons

Eqv to % NaCl	Mean	S. D	% haemolysis 95 % range
0.10	97	1 6	100.0—93.8
0.26	93	2.3	97.6—88.4
0.32	91	2.6	96.2—85.8
0.38	86	4.3	94.6—77 4
0.42	75	8.9	92.8—57.2
0.44	64	12.4	88.8—39.2
0.46	43	14.0	71 0—15.0
0.48	24	10.0	44.0— 4.0
0.50	9	4.5	18.0— 0
0.52	6	2.4	11.0— 0
0.54	3	1.5	6.0— 0
0.60	2	1.0	4.0— 0

Variation of PCV Variations were produced by adding or removing plasma from samples taken from the same person using whole blood as control.

Variations of the concentrations of Ca^{++} and glucose were produced by adding calculated amounts of $CaCl_2$ and glucose, untreated blood samples from the same persons acting as controls.

Measurements of the pH and standard bicarbonate were performed according to the method of Andersen et al. (1) The glucose and calcium concentrations were measured by conventional methods.

Results

The results of the determinations of osmotic fragility before and after incubation in 22 adult persons are given in table I Fig I demonstrates that it has been possible to obtain the same variability of the values of osmotic fragility before and after incubation at 37 C for 24 hours The important difference between the curves is the position relative to the abscissae the shape and width of the normal ranges being almost identical

The effect of the temperature during incubation appears from fig 2. The rise

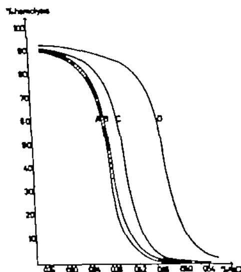


Fig. 2. The effect of variation of the temperature on the osmotic fragility after incubation for 24 hours. Blood from one person was used throughout the experiment.
A = 0 incubation; B = for 24 hrs at 5 C C = for 24 hrs at 25° C and D = for 24 hrs at 37° C.

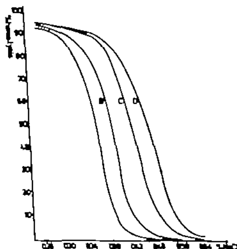


Fig. 3. The effect of varying the period of incubation at 37° C. Blood from six persons was examined. A, B, C, D represent experiments performed after 0—8—16—24 hours of incubation. Each curve represents the mean of the six samples examined.

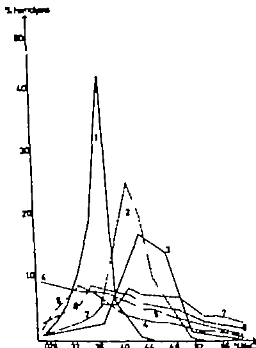


Fig. 4. The effect of varying the period of incubation from 0—72 hours. Curves 1 2, 3, 4 5, 6, and 7 represent experiments performed after 0—12—24—36—48—60 and 72 hours. The curves are increment curves constructed from the sigmoid curves by calculating the increment of haemolysis (%) obtained by lowering the osmolarity of the solutions equivalent to 0.02 % NaCl starting at the 0.60 % point.

in mean cellular fragility i e. the osmolarity giving 50 % haemolysis, seems to be exponential. However when the period of incubation is not exceeded, the general shape of the curves at 5—25—37° C has not changed which means that the distribution of relative fragility in the blood sample was not affected by a change of the temperature of incubation within these limits. The influence of the period of incubation is illustrated by fig 3. The increase in osmotic fragility during the first 24 hours seems to develop at an almost constant rate and affects the whole

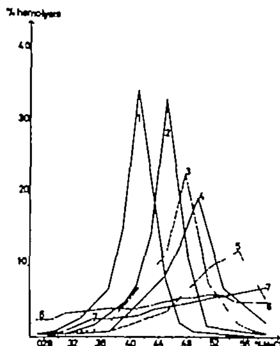


Fig 5 In this experiment the concentration of glucose was raised to secure substrate for continuation of the glycolysis for 72 hours. The experimental procedure was — apart from the addition of glucose — exactly the same as in fig 4 Blood from one person was used throughout the experiments depicted in fig 4 and 5

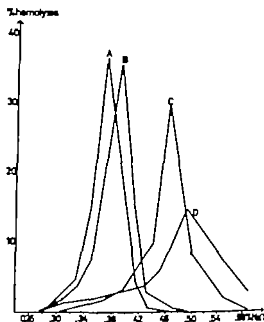


Fig 6. The effect of adding lactic acid or sodium carbonate to blood samples from one person before incubation at 37° C for 24 hours. The standard bicarbonate of untreated blood was 22 mEq/L. After the addition of acid the standard bicarbonate was 12 mEq/L and after the addition of base 36 mEq/L. A and B represent the course of haemolysis of alkalinized and acidified blood before incubation. Curve C = acidified blood and D = alkalinized blood after incubation for 24 hours at 37° C.

erythrocyte population simultaneously The effect of prolonging the period of incubation is shown in fig 4 The increment haemolysis curves clearly illustrate that an abrupt change of the osmotic fragility occurs after 24 hours of incubation the distribution of the osmotic fragility of the erythrocytes changing from a dome-shaped curve to a rectilinear This change is dependent on the glucose metabolism. Fig 5 shows how this change was delayed for more than 12 hours by the addition of glucose to a sample of the blood used in the experiment in fig 4 at the beginning of incubation

The effect of varying the pH of the blood appears from fig 6 The more alkaline the blood at the beginning of incubation the greater is the rise in red cell fragility after incubation. Acidification of a blood sample before incubation results in a smaller increase in fragility after incubation than with alkalinized blood. This is in agreement with the effect of the pH on blood storage (11)

Variation of PCV will only produce slight differences in the percentage of haemolysis obtained before incubation. Fig 7 shows the course of haemolysis with two samples of blood from the same person. In B 3 ml plasma was removed per 10 ml blood In A, 3 ml plasma was added per 10 ml blood resulting in a PCV in A of 67 and in B of 35 As may be seen from the figure only a small

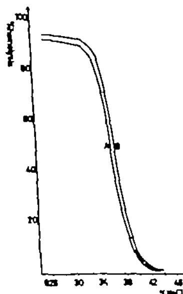


Fig. 7 The effect of variation of the packed cell volume on the course of haemolysis. Blood from one normal person was used in the experiment. Samples with PCV of 35 (A) and 67 (B) were produced as described in the text and osmotic fragility tests without incubation performed.

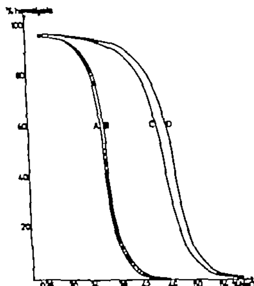


Fig. 8. With an experimental procedure as in fig. 7 blood samples with PCV of 37 and 57 were produced. A and B represent fragility tests carried out before incubation and C and D the fragility tests after incubation for 24 hours at 37° C (PCV 37 = A and C, PCV 57 = B and D).

change of th osmotic fragility was produced by the large difference in PCV With incubated blood however an even smaller difference in PCV will result in significant changes of the osmotic fragility In fig 8 curve A represents the course of haemolysis of blood with PCV 37 and B blood with PCV 57 before incubation nd curves C and D the same samples examined after incubation for 24 hours at 37° C.

Variation of the concentration of calcium by adding CaCl to a sample with a concentration of 10.4 mg % resulting in a concentration of 16.2 mg % did not affect the course of haemolysis relative to a control, neither before nor after incubation.

Variation of the concentration of glucose (glucose concentration 105 mg %) Glucose was added to a blood sample to a concentration of 305 mg %. The osmotic fragility test performed with the sample and a control sample before and after incubation at 37° C for 24 hours showed, that the addition of glucose resulted in a decrease of the osmotic fragility after incubation relative to the control as illustrated by fig 9

Fig 10 shows the fall of the concentration of glucose during some of the experiments reported above. The significance of the various rates of fall will be discussed below The non-glucose reducing substances of normal blood usually amounts to the equivalent of 15—20 mg % glucose. Thus the concentration of glucose of normal samples incubated at 37° C is actually about zero after 24 hours and has probably been so for at least 8 hours, as

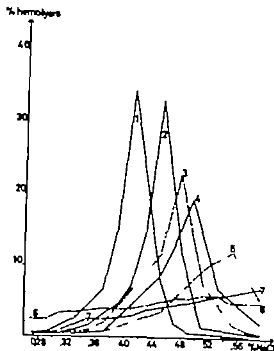


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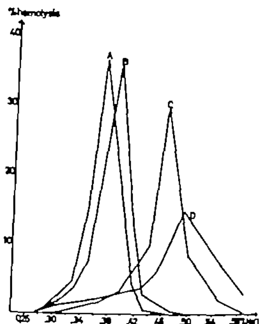


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with normal concentrations of glucose. Fig. 8 stresses the importance of correct ng the PCV by adding or removing plasma. The recently reported relati e decrease of osmotic fragility after incubation of erythrocytes from patients with ron deficency (8) may thus be an artefact as blood specimens with very low PCV values are compared with normal blood without any correction.

The effect of these factors may be exerted via the glucose metabolism of the red cells. A rise of the temperature of incubation accelerates glycolysis. The glucose content of the erythrocytes will quickly be exhausted during the incubation period and the lack of substrate for glycolysis might increase the fragility of the red cells. The actual glucose concentrations determined after incubation at 5—25 and 37 C (see fig. 10) support this iew A similar effect might be produced by changes of the pH, as the optimal pH for glycolysis is about 8.1 the glycolytic rate decreasing rapidly with decreasing pH of the blood The concentrations of glucose during the ex periments depicted n fig 6 may be seen from fig 10 The effect of variations of the PCV may be explained in a similar way with a low PCV the amount of glucose/ml erythrocytes will be higher than in blood with a high PCV value

With the osmotic fragility test applied in this work the variation of fragility in normal persons before incubation and after incubation is of the same magnitude (fig 1) Moreover the distribution of relative fragility throughout a normal erythrocyte population is not altered by incubation at 37° C for 24 hours. Fig 4 illustrates that a sudden change of the distribution of the relative fragility occurs, when the period of incubation is prolonged. In fig. 5 this change has been delayed by adding glucose. This suggests, that continued glycolytic activity is essential for the characteristic distribution of osmotic fragility in a normal erythrocyte population.

The course of haemolysis when erythrocytes are exposed to a series of hypotonic solutions is represented by the well known sigmoid shaped curve, from which a curve representing the distribution of relative fragility of the erythrocyte population can be constructed. As shown by Maizels 1949 (11) and later confirmed by Selwyn and Dacie 1954 (21) the preservation of the red cell cation composition during incubation depends upon the presence of glycolysis, which probably supplies the energy for the active cation transport. This seems to justify the assumption that the distribution of fragility in a normal erythrocyte population may depend upon the relative efficiency of the active cation transport mechanism in opposing the passive ion movements, which are determined by the concentration gradients. Differences in the efficiency of the active cation transport of the individual cells might be brought about by variations in the activity of the adenosine triphosphate splitting enzyme system which seems to be essential to or identical with the active transport mechanism (18) or by differences of the ATP level and ATP regenerative power of the cells, which are dependent on the glycolytic activity of the individual cells.

Studies of differences of the ATP splitting enzyme activity of cell groups of varying osmotic fragility have not yet been performed.

Investigations into the in vivo and in vitro geing of human erythrocytes have demonstrated that a decrease of the activity of glucose-6-phosphate dehydrogenase and the 3-phosphoglyceral-

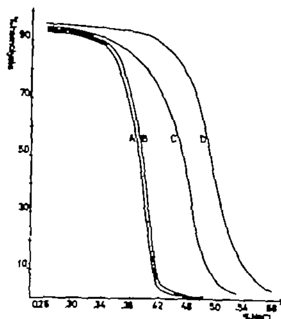


Fig 9 The effect of adding glucose to blood samples before incubation. A and B represent the fragility tests before incubation and C and D the fragility tests after incubation t 37 C for 24 hours. A and D represent normal blood and B and C samples of the same blood with glucose added to a concentration of 300 mg

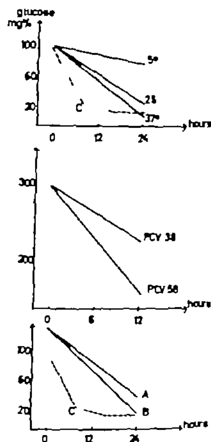


Fig 10. The glucose concentration during incubation at different temperatures (top) and during incubation of blood with different PCV values (middle) and different pH values (bottom) Curve C represents the fall f the glucose concentration during the experiments in fig 3 and shows the normal course of the glucose concentration during incubation for 24 hours at 37° C.

may be seen from curve C in fig 10 This curve represents the fall in the concentration of glucose during the experiments depicted in fig 3 The concentration of glucose of the alkalinized blood sample has also reached the zero level, when determined after incubation for 24 hours at 37 C. This tends to underestimate the glycolytic rate of blood samples at 37 C to which base was added

Discussion

The results of the experiments reported in this paper indicate that certain variable factors must be carefully controlled during the incubation-fragility test. The effect of the temperature changes is so extreme within the 37 C range that thermostatic control is necessary The period of incubation should be strictly fixed at 24 hours, as the sudden changes of fragility which occur after this time will make comparisons with 24-hours-values impossible. The pH of the blood should be within the normal ranges as even clinically occurring variations of the pH of the blood may cause significant changes in the results of the test. The glucose concentration should be normal at the beginning of the incubation as important decreases of the osmotic fragility may result from an elevation of the blood sugar relative to the fragility of samples

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dehydrogenase is closely correlated with cell ageing and destruction (10) Concomitantly with the fall of the activity of the 3 phosphoglyceraldehydrogenase the concentration of ATP is diminished The active cation transport mechanism which seems to depend upon the above mentioned ATP-splitting enzyme system might thus be varying with the age of the erythrocytes and explain the well established variation of the osmotic fragility of normal human erythrocytes with the cell age (12 13 22)

The decrease in activity of the glucose 6-phosphatdehydrogenase may also be of great importance for the ageing of the erythrocytes During recent years it has been demonstrated that individuals whose erythrocytes are deficient of this enzyme are apt to develop a haemolytic anaemia when exposed to various drugs (4 7 25) The haemolytic mechanism is not known in detail but a consequence of a defective hexosemonophosphate shunt is the formation of oxidized glutathione and methaemoglobin (11) Glutathione is known to be necessary for the triosephosphatdehydrogenase reaction and may in this way influence ATP regeneration (9)

The importance of glycolysis for the viability of the erythrocytes is also demonstrated by the pyruvate-kinase deficiency of the red blood cells of certain patients with haemolytic anaemia (14 24)

That ATP regeneration and ATP concentration is closely correlated with erythrocyte shape and active cation transport has been shown by several investigators during incubation experiments with stored erythrocytes (15 17 20 23) Incubation with adenine and inosine after storage for several weeks at 4 C resulted in ATP regeneration and change of erythrocyte shape from sphere to disc (17) and regeneration of active cation transport (15 20) The survival of cells transfused after incubation with adenine and inosine was considerably prolonged compared with control cells (6 17)

Various investigators have failed to show any decrease of the osmotic fragility after incubation with glucose added to the blood as compared with the fragility of samples of the same blood without the addition of glucose (16 17) Fig 9 shows that with the present author s technique of osmotic fragility test the addition of glucose during incubation definitely reduced the fragility of these erythrocytes relative to the control blood sample. The experiment depicted in fig 9 is a representative of 4 experiments all showing the same effect of adding glucose to the blood prior to incubation

The increasing knowledge of the biochemical pathogenesis of various types of haemolytic anaemia will probably result in standardized specific tests as for instance the glutathione stability test (2) the pyruvate kinase test (24) and others. The vast experience with the osmotic fragility test, however will secure its continued use as a screening test which may yield valuable information particularly when performed under standardized conditions.

Summary

The increase of osmotic fragility of the normal human erythrocytes during incubation is greatly influenced by the temperature and time of incubation of the pH and the concentration of glucose of the blood prior to incubation and of the ratio erythrocytes/plasma of the blood

On the basis of observations in 22 healthy adult persons normal values of the osmotic fragility after incubation at 37° C for 24 hours are given

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Studies on the Osmotic Fragility of Normal Human Erythrocytes

IV A Method for the Determination of the Effect of Changes in pH on the Fragility of Erythrocytes ("pH Fragility Test")

By

Leifer Mortensen

Knowledge of the relation between hemolysis and the pH of hemolytic solutions originates from the pioneer work of Hamburger (5) about 60 years ago Hamburger demonstrated that increased acidity of a hemolytic system caused increased osmotic fragility of red blood cells, regardless of whether the increased acidity was brought about by adding CO_2 or a non-volatile acid. Furthermore Hamburger associated this effect of acid with a change in the base-binding capacity and osmolar effect of the intracellular proteins. This view was supported and expanded by the investigations of van Slyke et al. (15) and of Warburg (14) who succeeded in establishing quantitatively the relation between pH and red cell volume. Jacobs, in an important paper published in 1931 (6) examined the relation between pH and hemolysis, and by fitting his own figures into the equations put forward by van Slyke et al. he succeeded in demonstrating a similar relation between acidity and hemolysis.

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Though Jacobs clearly demonstrated the great influence of pH upon the course of the hemolytic process, it was not until 1947 that an osmotic fragility test which took account of the pH was elaborated by Parpart et al. (11) As demonstrated in a previous paper this method entails an important error resulting in variability of the pH of the hemolytic solutions (10)

The study of the quantitative importance of the pH made possible the elaboration of a fragility test in which small variations are made in the pH of the hemolytic solutions.

Details of the method will be described and normal values given and compared with the results of an osmotic fragility test performed on the same samples of blood

Material

Blood from healthy volunteer blood donors was used. Twenty persons aged between 18 and 55 were examined. None of the persons

Table II. The composition of the hemolytic solutions. Ionic strength = 0.085 pK = 6.76 osmolarity = 0.157

I	II	III	IV	V	VI	VII
pH	Na_2HPO (mMol/l)	NaH_2PO (mMol/l)	NaCl (mMol/l)	Na_2HPO (g/l)	NaH_2PO (g/l)	NaCl (g/l)
6.60	10.00	14.460	38.94	1.7800	1.9855	2.2760
6.80	10.00	9.116	44.29	1.7800	1.2580	2.5888
7.00	10.00	5.754	47.75	1.7800	0.7940	2.7910
7.20	10.00	3.631	49.87	1.7800	0.5011	2.9149
7.40	10.00	2.291	51.21	1.7800	0.3161	2.9932
7.60	10.00	1.446	52.06	1.7800	0.1995	3.0429
7.80	10.00	0.912	52.59	1.7800	0.1258	3.0739
8.00	20.00	1.150	57.35	3.5600	0.1449	2.1831
8.20	20.00	0.662	58.24	3.5600	0.0914	2.2058

The concentration was doubled in order to increase the buffering capacity

room air the procedure for standardization has been described elsewhere (10). 50 μl blood was added to 5,000 μl of each of the hemolytic solutions employed. All experiments were performed within few hours of the venipuncture.

The time

of hemolysis was extended to 120 min., which control experiments had shown to be sufficient for the hemolytic process to each equilibrium at 10° C.

The hemolytic solutions

The osmolarity permits only small buffering capacity of the solutions even if most of the NaCl is replaced by NaH_2PO_4/Na_2HPO. An amount of 10 mMol/l of Na_2HPO was arbitrarily fixed and the concentrations of NaCl and NaH_2PO at each pH level were calculated by means of the usual buffer equation

$$pH = pK + \log \frac{C_B}{C_A}$$

The pK value at the ionic strength 0.085 is = 6.76. The value is calculated by means of the Debye—Hückel equation.

The substitution of phosphate ions for chloride ions may introduce another variable factor since the chloride ion has been shown to pass the erythrocyte membrane more quickly than the phosphate ions (12). An evaluation of the importance of this difference is, however, not possible since the pH must be the same in a comparison. The composition of the hemolytic solutions which allows the pH to be varied from 6.6—8.2 appears from table II. Although the amount of blood added is small, the buffering capacity of the blood is great enough to produce shift of the pH of the mixtures of blood and hemolytic solutions, *but the shift will be constant from experiment to experiment provided that the buffering of the blood applied has been standardized.*

The substance mainly responsible for the buffering capacity of the blood when the CO_2/HCO_3 has been abstracted is hemoglobin. The buffering capacity of hemoglobin depends upon the degree of oxygenation, the amount of carbhemoglobin, the temperature and the ionic strength (9). With these factors standardized constant shift of the pH will be achieved, and the pH of the hemolytic mixtures measured after 120 min. at 10° C will show good constancy. In table III the variability of the pH at all levels of all the samples examined is recorded.

Thus anemia will influence the degree of hemolysis by the effect of hemoglobin on the pH and the effect of the added blood on the osmolarity of the solutions. In standardizing anemic blood the erythrocytes will shrink more than normal (3) and this too will affect the results if not corrected for. The simplest way of correcting for the influence of anemia

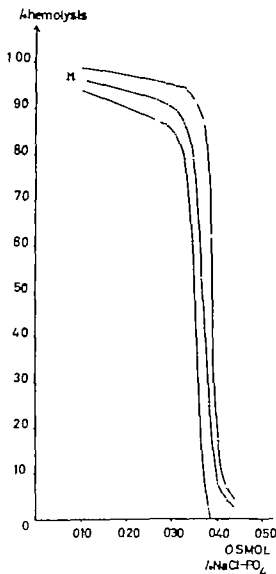


Fig 1 The normal values of the osmotic fragility test performed concomitantly with the pH fragility test. The mean and 95 % range is shown.

had been used as a donor for the last 6 months before the examination or suffered from acute illness. To confirm the clinical impression of the healthy state of the donors, the following tests were routinely performed on all blood samples determination of hemoglobin, erythrocyte count, leucocyte count, reticulocyte count, determination of the hematocrit, E.S.R. serum bilirubin, serum creatinine, plasma iron and iron binding capacity No person had to be excluded from the material because of deviation from the normal ranges.

Table I The laboratory tests applied and their normal range (95 %)

Determination of	Method applied	Normal range
Hemoglobin	Drabkin	The hemato-logical values are identical with those of Wintrobe (15)
Erythrocyte count	Conventional	
Leukocyte count	Conventional	
Reticulocyte count	Conventional	
Hematocrit	Micromethod	
ESR	Westergren	2—10 mm/h
Serum bilirubin	Jendrassik & Grof	< 1.0 mg%
Serum creatinine	Bosnes & Taussky	< 1.3 mg%
Plasma iron	Sobel & Chiamori	5.–198 µg%
Iron-binding capacity of plasma	Sobel & Chiamori	257–379 µg%

Methods

The methods and normal values of the routine tests are shown in table I

The osmotic fragility test applied has been described in detail in a previous paper (10) The normal values appear from fig 1

If the various factors influencing the hemolytic process are kept constant except the pH, which is systematically varied, the "pH fragility-test" will result. A detailed discussion of the importance of these factors has been given previously (10)

Temperature

was fixed at 10° C (maximal deviation 0.5 C)

Osmolarity

All the solutions applied had an osmolarity equal to an 0.40 NaCl-solution. (= 0.137 osmol.)

The blood

was obtained as heparinized venous blood and was standardized at 100 % oxygen saturation and a CO_2-tension equal to that of

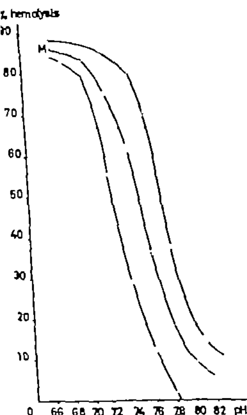


Fig 2. The normal values of the pH-fragility test elaborated. The mean and 95 % range is shown.

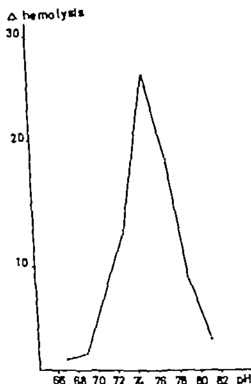


Fig. 3. The distribution curve of hemolysis for the pH resistance test. The increments of hemolysis were calculated from fig 2.

The preparation of the mixtures

The reagents used are
NaCl (Merck p.a.)
$Na_2HPO_4 \cdot 2H_2O$ (Merck p.a.);
$NaH_2PO_4 \cdot H_2O$ (Baker p.a.)

The samples were weighed on an analytical balance and dissolved in redistilled water in volumetric flasks. The pH of the solutions was controlled after the preparation and once a week during use. The ranges of the values appear from table IV.

The pH measurements were performed with glass electrode using an NBS-certified buffer as reference.

The precision of the hemolytic method expressed by the variability of duplicates appears from table V.

Results

The normal values are recorded in fig 2 and table VI. The increment of hemolysis for alterations of the pH of the hemolytic mixtures may easily be calculated from fig 2 and plotted on the ordinate in relation to the pH values on the abscissa. Such a graph represents a distribution curve for hemolysis (fig 3).

The results of the concomitantly performed osmotic fragility test were all within the normal range of the method (fig 1). In order to elucidate whether a deviation in pH-resistance for a given sample was correlated with changes in the osmotic fragility test, a scatter dia

Table III. The pH values of the hemolytic mixtures after 120 min. at 10 C The figures were calculated on the basis of 20 pH resistance tests

pH of solution	pH of mixture mean	S. D.	Mean ± 2 × S D (95 % range)
6.6	6.69	0.011	6.67–6.71
6.8	6.89	0 009	6.87–6.91
7 0	7 07	0.011	7 05–7 09
7.2	7.25	0.011	7.23–7.27
7 4	7 43	0.012	7 41–7 45
7.6	7.59	0.020	7.55–7.63
7.8	7 74	0.024	7 69–7 79
8.0	7.96	0.023	7.91–8.01
8.2	8.05	0.025	8.00–8.10

Table IV The pH of the hemolytic solutions Control measurements were performed after preparation and once a week during use

pH calculated	pH — range measured
6.60	6.62–6.65
6.80	6.80–6.85
7.00	6.99–7 05
7.20	7.20–7.23
7 40	7 40–7 43
7 60	7.58–7.63
7.80	7.80–7.83
8.00	7.98–8.04
8.20	8.18–8.23

Table V The variability of the method expressed by the variability of duplicates. The values (95%) were calculated from 30 consecutive pairs of values of the optical density for the ranges 50—100% and 0—50 % of hemolysis

	50–100 %	0–50 %
Mean	205	39
S. D	1.35	1 13
S D. × 1 4	1.89	1.58
Coefficient of variation	0.92	2.90

Table VI pH-resistance-test Normal values (95 %) The figures were calculated on the basis of 20 experiments

pH of hemolytic solution	Percentage of hemolysis		
	Mean	S. D.	Mean ± 2 × S. D.
6.7	86	1	88–84
6.8	85	1	87–83
7 0	83	2	87–79
8.2	75	10	95–55
7 4	62	16.5	95–29
7 6	36	17	70 2
7.8	18	10	38– 0
8.0	9	5	19– 0
8.1	7	4	15– 5

80 % hemolysis, pH 6.96–7 46.
50 % hemolysis, pH 7.24–7 74.
20 % hemolysis, pH 7.50–8.00.

will probably be the adjustment of the hematocrit to normal value by removing some of the plasma. Only normal blood was used in the study

Practical procedure

All tests should be carried out in duplicate. Centrifuge tubes containing 5 000 μl of the hemolytic solutions of pH 6 6—6.8— 8.2 and redistilled water are adjusted to 10° C. Heparinized blood obtained at venipuncture is standardized and 50 μl of the standardized blood is added to each tube. The tubes are stoppered and gently homogenized by inversion at once and every 15 min. during the experimental period 120 min. After centrifugation at 1,500 × G for 3—5 min. the hemoglobin concentration of the supernatant fluid in the tubes is determined by pipetting 2 000 μl into 5,000 μl Drabkin-solution. The optical density is determined with a photoelectric colorimeter at 574 nm. The pH of the hemolytic mixtures is measured at the end of the hemolytic period.

The per cent hemolysis is calculated by dividing the optical density of the solutions by the density of the tube with distilled water and multiplying the ratio by 100. The % hemolysis in distilled water is hereby defined as being 100 The results are plotted in a rectilinear coordinate system with the hemolysis as ordinate and the pH as abscissa.

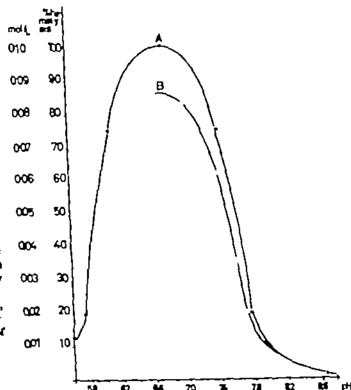


Fig. 5 The relation between buffering capacity and pH of the solutions (curve A) and between % hemolysis and the pH (curve B).
Curve B is identical with the mean from fig. 2.
Curve A was calculated by means of the equation:
$\beta = 2.303 \times C \times \quad (1 + \quad)$, where β = buffering capacity C = molar concentration of acid + base and $\quad = \frac{C_{base} + C_{acid}}{C_{base}}$

hemoglobin content of the blood sample within the normal range. The variability of the pH measurements is below $\pm$ 0.01 of a pH unit.

The variability of the pH of the hemolytic mixtures is greater at a pH of about 8 than at a level of 7 This is due to the rapid decrease of the buffering capacity outside the range of the pK $\pm$ 1 pH unit. In this method the pH is varied from the pK and upwards in order to secure that the highest % of hemolysis will be accompanied by the greatest buffering capacity the liberated hemoglobin being the most important factor which affects the pH constancy This is illustrated by fig. 5 where the relation between buffering capacity hemolysis and the pH has been recorded.

Only one method of testing the pH resistance of red blood cells has previously been published (7) Van Kampen et al. developed a method in which variations of the pH were produced by changes in the components of the citric acid/Na_2HPO_4-buffering system. The pH of the solutions was varied between 3.9 and 5 7 The authors found with this method some very impressive deviations from their normal values in disease states where the osmotic fragility test yields normal values Unfortunately the method shows important errors which makes the results questionable. Unstandardized blood is applied and the ratio blood/solution is high 100/2,000 whereby a great variability of the pH values is produced — on the average more than 0.2 of a pH unit for

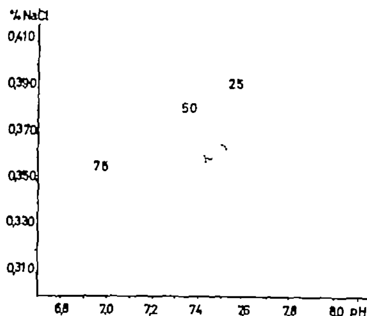


Fig 4 Scatter-diagram showing the correlation between the pH fragility test and the osmotic fragility test for the hemolytic levels 25—50—75 % for normal blood.

gram for the levels 25—50—75 % of hemolysis was constructed (fig 4) The coefficient of correlation was calculated *for the 50 % level* and was found to be 0 98 Thus for normal persons changes in the pH resistance will be closely correlated with changes in osmotic fragility

Discussion

In elaborating this fragility test three important questions arise Has the test been correctly developed? Will the test make any existing method superfluous? Will the existing tests supersede this test? The basic demand on a fragility test like this must be that it actually varies the pH of the hemolytic mixtures while at the same time the other factors influencing hemolysis are kept constant For a discussion of these factors and the methods for keeping them constant during the hemolytic process the reader is referred to a previous paper (10)

The variations of the pH selected for this method are under given conditions capable of producing changes in the percentage of hemolysis from 0—90 %. With temperature higher than 10 C or an osmolarity higher than 0 137 osmol, smaller percentages of hemolysis would have been obtained. With a lower temperature or a lower osmolarity higher percentages of hemolysis would have been found. The numerical changes of the dependent variable, i. e. the hemolytic process, will be sufficiently large to characterize the independent variable, i. e. the pH of mixtures, account being taken of the precision of determination of the degree of hemolysis (table V) and of varying the pH (table IV) It has not been possible to produce equal changes of the pH from tube to tube, but the distribution of the pH values suffices for the characterization of the hemolytic curves. The reproducibility of the particular levels of the pH is sufficient for the purpose. The variability of the pH levels is caused by several factors variations of the reagents, the weighing and measuring of the reagents and solutions during preparation and during the practical procedure of the test, and variation of the

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Blood-volume During Treatment of Hypertension with Guanethidine

By

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During treatment of hypertension with ganglion blocking agents, weight gain due to retention of salt and water has been described. This is sometimes followed by considerable fall in haemoglobin values, and manifest heart failure has occurred (2,6). Later it was found that in most hypertensives there was a rise in blood volume during treatment with ganglion-blocking agents (7, 9). A few years earlier Smith and Hoobler (10) had been unable to demonstrate significant changes in blood-volume during treatment with pentolinium.

In most patients this weight gain and retention of salt and water was moderate. During treatment with guanethidine things are different. Weight gain and peripheral oedema are often seen. Dollery et al. (1) treated 80 patients and six gained 10 lb or more. Four of them developed congestive heart failure. Galakov et al. (3) state that heart failure similarly occurred in 6 of their 42 cases, and in 5 cases the treatment had to be abandoned. Leishman et al. (5) report that many patients gained 2 kg or more during treatment. In 17 of 114 cases weight gain was accompanied by increasing breathlessness. In a few cases this was associated with raised jugular venous pressure and peripheral oedema.

To throw more light on these facts, repeated blood-volume determinations have been carried out in a series of hypertensive persons during treatment with guanethidine. Although the investigation has not yet been finished it may be of value to report the first series of results here.

Material and methods

The principle of the study has been to measure the blood-volume before and during treatment with guanethidine.

Twelve ambulatory patients were studied. Nearly all had severe hypertensive disease, 3 being in malignant phase. None showed signs of cardiac failure. During the trial the patients were on a diet containing 6—7 g sodium chloride a day. Fluid intake was unrestricted. The patients were treated with guanethidine, the dose varying from about 15 to 75 mg daily.

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the 95 % range. In spite of the low temperature during hemolysis the hemolytic time is fixed at 30 minutes which is less than the time necessary for equilibrium (11). The estimation of the degree of hemolysis is done by visual colorimetry which is too inaccurate for determining the small increments of hemolysis at high and low percentages of hemolysis. The osmotic fragility test with which the method is compared (2) overlooks the importance of pH-constancy and uses visual colorimetry and moreover the blood and solutions are measured in drops instead of well defined volumes. The most important objection which can be raised against the method of van Kampen et al. is that the osmolarity is not kept constant during the test. The percentages of hemolysis obtained are the results of changing osmolar and pH conditions. Whether the effect of the high H^+ ion concentrations is the result of a change of the erythrocyte membrane as proposed or whether it is produced by changes of the base binding capacity of the red blood-cell proteins cannot be settled. Other investigators have found an even greater variability of the normal values (1) using the method of van Kampen et al.

Only normal blood was used during this investigation. The use of the test in cases with enhanced hemolysis cannot be predicted. In the Marchiafava-Micheli syndrome the finding of enhanced hemolysis, normal osmotic fragility test and increased fragility when the pH of the blood is lowered (Ham's test (4)) indicates that a pH fragility test may reveal changes of the properties of the red blood cells even if the osmotic fragility test yields normal values. Whether this feature is restricted to this rather rare syndrome or may be found in other states of disease cannot be predicted. The high degree of correlation between the osmotic and the pH fragility tests for normal blood does not preclude that diagnostically significant information may be obtained in various types of anemia.

Summary

The fragility of the normal human erythrocyte is greatly influenced by the pH of the surrounding medium. Under suitable conditions a variation of % hemolysis between 0 and 100 may be obtained with small variations of the pH. On this basis a pH fragility test for human erythrocytes has been elaborated. The method is discussed and normal values obtained in 20 healthy persons are given.

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re prepared from this solution immediately fore it was injected intravenously. The antity injected was calculated by weighing e syringe before and after the injection. uring the following 40 minutes 3 blood mples were drawn without stasis, the first ie 10—15 minutes after the injection. After ntrifugation plasma samples were prepared duplicate.

Activities of standards and plasma were easured in a well-type scintillation detector lasma taken just before the injection was sed as plasma blank. Counting of the plasma amples was continued until the standard leviation of the difference between sample and blank was less than 1 per cent. The loss rate or plasma activity will exhibit a constant slope when plotted on a semilogarithmic time-activity scale. Back-extrapolation to the injection time will give the value for plasma activity corrected for loss (4). Plasma volume was calculated as the ratio between injected activity and the increase of activity per ml of plasma so determined.

All patients were weighed every day and blood-pressure was measured in erect and supine position 3 times a day.

In patients nos. 1—7 blood-volume determinations were done in the afternoon, and in each case the patient had been kept in bed for at least 3 hours before the trial. In patients nos. 8—12 blood-volume determinations were done before lunch and the patients had to stay in bed until the test was finished. In each individual case each trial was done at the same time of the day to ensure uniform experimental conditions.

Results

Table I shows the effect of the treatment on blood-volume weight and blood-pressure in the 12 patients. The blood-volumes were determined before and after 7—21 (on an average 13) days of treatment with guanethidine. The volumes increased in all cases, the difference varying between 80 and 750 ml with a mean of 437 ml. Standard error $\pm$ 55 ml $p < 0.001$. The difference between blood volumes before and during treatment is consequently highly significant.

In most cases there was an increase in weight (maximum 2.2 kg) but in three cases there was a slight decrease (table I). The mean weight gain was 0.85 kg $\pm$ 0.335 kg. The difference is significant ($p < 0.05$). However these results need comment. Two of the patients had intercurrent diseases which might be expected to reduce the weight between the two blood-volume determinations. Furthermore some of the patients voluntarily reduced the daily food intake when they discovered the weight gain, and it was difficult to persuade them to take their usual amount of food. During the treatment one patient (no. 12) experienced increased breathlessness, and a few moist râles were heard over the lungs. In two patients (no. 7 and 12) slight facial oedema was observed. In no case was dependent oedema found.

Discussion

As mentioned above, weight gain on account of retention of salt and water is often seen during treatment of hypertension with guanethidine. This study shows that the treatment furthermore is accompanied by a considerable increase in blood-volume. Preliminary results of further studies (to be published later) seem to indicate that increases in extracellular space and total exchangeabl sodium also occur.

It is interesting to note that in only one of 12 patients was the increase in blood volume (on an average nearly 10 per cent) and the fluid retention accompanied by signs of slight heart-failure. As a rule the fluid retention can be satisfactorily counteracted by simultaneous treatment with nonmercurial diuretics.

The cause of these phenomena is still unknown. In earlier work (9) I suggested

Table I Blood-volume, weight and blood pressure (supine and erect) before and during treatment with guanethidine

Case no.	Blood-volume (ml) and weight (kg)		Difference	Blood pressure (supine and erect)	
	Before treatment	After 7—21 days of treatment		Before treatment	During treatment
1	5,920	6,380	+ 460	190/110	180/100
	73.5	74.5	+ 1.0	155/110	160/ 95
2	4,860	5,310	+ 450	170/115	140/ 95
	60.0	61.5	+ 1.5	160/110	125/ 90
3	3,800	4,340	+ 540	210/120	175/105
	54.5	55.5	+ 1.0	155/110	125/ 85
4	3,530	3,800	+ 270	160/100	140/ 85
	59.5	?	?	145/100	135/100
5	4,530	4,880	+ 350	235/115	190/105
	92.6	91.5	— 1 1	230/125	160/ 95
6	5 620	6,210	+ 590	175/120	145/ 95
	80.6	81.8	+ 1.2	190/135	125/ 85
7	4,590	5,290	+ 700	215/145	185/115
	68.2	70 4	+ 2.2	195/150	165/110
8	4,620	4,920	+ 300	175/110	155/ 95
	91.5	91 1	— 0 4	155/110	110/ 80
+9	2,810	2,890	+ 80	235/135	230/115
	48.0	47 4	— 0 6	185/130	125/ 90
10	5 530	6,280	+ 750	190/115	165/110
	86.0	88.0	+ 2.0	175/120	155/105
11	4 850	5,200	+ 370	170/115	160/110
	66.2	66.8	+ 0.6	155/110	135/ 85
12	4,640	5,020	+ 380	230/140	205/125
	67.2	69 1	+ 1.9	230/155	190/125

Mean increase in blood-volume 437 ml. S. E. ± 55 ml $p < 0.001$
Mean increase of weight 0.85 kg. S. E. ± 0.335 kg $p < 0.05$.

Sympathectomized more than 10 years ago.
Bronchitis with fever most of time between blood-volume determinations.
Treatment with guanethidine caused gastric discomfort and anorexia.

Total blood-volume was calculated as the sum of erythrocyte-volume and plasma-volume.

Blood-volume was determined by means of radioactive sodium chromate (for the technique employed see Rønnov Jensen (7)). To calculate red cell volume, the blood-volume was multiplied by the mean value from duplicate determinations of venous haematocrit.

The haematocrit values were corrected for 4 % trapped plasma.

Measurement of *plasma-volume* was carried out by means of ^{131}I-albumin in direct continuation of the former procedure. 10—17 μC ^{131}I-albumin was dissolved in about 10 ml of the person's own plasma in order to minimize the absorption of the radioactive iodinated albumin on glassware. Standards

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The Symptomatic Effect of Anticoagulant Therapy in Defibrination Syndrome Associated with Demonstrable Fibrin in Plasma

A Case Report

By

H. C. Godal and U. Abildgaard

This paper concerns a patient suffering from carcinoma of the pancreas. Terminally signs of intravascular coagulation (hypofibrinogenaemia, thrombocytopenia, low plasma values of antihaemophilic globulin (AHG) and proaccelerin, bleeding tendency) were observed. In addition, small amounts of fibrin could be demonstrated in the plasma. The fibrinaemia and the other signs of hypercoagulation disappeared during treatment with heparin whereas phenylindandione (PID) was ineffective in this respect.

Case report

The patient was 70-year-old woman. There was no record of earlier bleeding tendency. Since early in spring, 1962, she had suffered from dorsal and abdominal pain, fatigue, anorexia and loss of weight. In August, 1962, cutaneous haemorrhages were noticed.

On September 4th, 1962, she was admitted to Krohgstøtten Hospital, Oslo, with signs of cerebral haemorrhage. On admission, she was somnolent, and there was paresis of the right leg. The cerebro-spinal fluid was blood-tinged. Large ecchymoses were found on the legs. No abdominal tumour could be palpated.

Laboratory findings. Haemoglobin 13.6 g %, ESR 15 mm/1 hour. Leucocytes 10 800/mm³ with normal distribution. The prothrombin level (P-P method of Owren and Aas) was 54 % and the number of thrombocytes 152,000/mm³. The bleeding time was prolonged, more than 30 min. The sternal marrow was normal, as were X-ray examination of the chest and the stomach. There was a slight proteinuria, and positive benzidine reactions in the faeces.

During hospitalization, her mental condition improved, and the subcutaneous haemorrhages subsided spontaneously.

Plasma, examined at the Institute for Thrombosis Research, Rikshospitalet, Oslo, revealed low values of fibrinogen (0.125 g/100 ml) of antihaemophilic globulin (27 %), and of proaccelerin (33 %). There was no evidence of fibrinolysis. Based on

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that the retention of salt and water and rise in blood volume during treatment with ganglion blocking agents could be conditioned by an over production of aldosterone on account of reduced pulse amplitude. Table I shows that the present study yields no support to this hypothesis, as there has been no reduction in pulse amplitude during treatment with guanethidine.

During treatment with pentolinium, hexamethonium and pempidine an inverse relation between blood volume and sensitivity to these drugs has been demonstrated (7 8) The studies were carried out by measuring blood volumes and the reduction in blood pressure caused by a fixed intravenous test dose of the ganglion blocking agents in question before and during treatment.

A similar study during treatment with guanethidine would be of considerable interest, *especially in the initial stages* because the greatest fluctation in blood volumes undoubtedly are seen in the first weeks of the treatment. Unfortunately this cannot be done, because the gradual onset of the effect and the slow elimination of this substance will make it difficult to estimate the effect of an intravenous dose even before continual treatment is started. During treatment evaluation will be impossible on account of the pronounced cumulative effect of the previous doses.

Summary and conclusion

Twelve patients *with severe hypertension* were treated with guanethidine. In all cases an increase in blood volume resulted *This increase* was highly significant ($p < 0.001$) and rather considerable, on an average *nearly 10 per cent.* Weight gain presumably due to fluid retention was recorded in most cases.

This increase in blood volume and fluid retention is without doubt responsible for the development of heart failure occasionally seen during treatment with guanethidine. In this series one of twelve patients developed signs of heart failure.

Clinical observations suggest an inverse relation between blood volume and sensitivity to guanethidine (5) but the characteristics of the drug i e. gradual onset of the effect on blood pressure and very slow elimination make it impossible to confirm or disprove this theory by experimental investigation

Acknowledgement

I should like to express my thanks to Dr C. B. Madsen for permission to carry out the isotope measurements in the Radiophysical Department of the Radium Centre for Jutland, Århus.

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ce chest) a thready viscous precipitate was formed within 15—60 minutes. In some instances even a coagulum was formed. Occasionally precipitation occurred at room temperature. The precipitate was only partly soluble on re-warming to 37° C. It was insoluble in 5 M urea, but could be dissolved completely in alkaline urea (40 % urea in 0.2 N NaOH) and also by the action of plasmin (on incubation with streptokinase)

These observations do not present conclusive evidence as to the nature of the substance studied, but they strongly suggest that minute amounts of fibrin were involved.

Precipitation occurred even if precautions were taken to prevent coagulation *in vitro* (the samples were collected in plastic tubes, and centrifuged immediately) In the presence of heparin, precipitation occurred even more rapidly than if sodium citrate was used as anticoagulant. It can therefore be concluded that if precipitation was due to fibrin, this must have been present in the circulating blood.

Discussion

The so-called defibrination syndrome is occasionally observed as a complication to various diseases, such as carcinoma (especially prostatic and pancreatic (5)) obstetric complications (6) large haemangiomata (1) and is probably the dominating factor in purpura fulminans (2) Clinically the bleeding tendency is characteristic. On examination of the blood, the following components are lowered Fibrinogen, thrombocytes, AHG, and proaccelerin. In addition, fibrinolytic activity is often demonstrable (9) The changes are considered to be the result of intravascular coagulation.

The findings in our patient fit well with this syndrome. In addition, a substance present in plasma, insoluble in the cold, and only partly soluble on re warming was demonstrated. Most likely this substance represents a mixture of fibrin and fibrinogen, some of the fibrinogen being soluble on re-warming This view is supported by experimental work. Thus, Shainoff and Page (7) demonstrated that following incubation of purified fibrinogen with minute amounts of thrombin, coprecipitation of fibrin and fibrinogen occurred in the cold. Identical results are obtained if instead of purified fibrinogen normal human plasma is used (Godal and Abildgaard, to be published) The existence of preformed fibrin in the plasma of our patient is also supported by the demonstration of multiple thrombi and fibrin deposits at autopsy

In contrast to PID heparin strongly inhibited the defibrination process, as judged from the effect on plasma fibrinogen and the thrombocyte number We do not know the reason for this discrepancy but liberation of trace amounts of trypsin from the pancreatic tumour might have been a pathogenetic factor in the defibrination in this case. In the presence of minute amounts of trypsin, probably only proaccelerin (factor V) is necessary for the conversion of prothrombin to thrombin (8) Therefore, reduction of factor VII factor IX and factor X by treatment with PID will be of little if any value. On the other hand, heparin being a potent antithrombin will prevent fibrin formation irrespective of the mechanism by which prothrombin is converted to thrombin.

Summary

A patient suffering from pancreatic carcinoma associated with defibrination syndrome is reported. Evidence of preformed fibrin in plasma is presented.

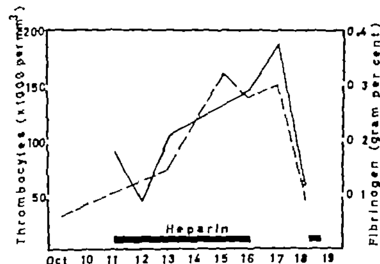


Fig 1 Effect of heparin treatment on plasma fibrinogen (continuous line), and thrombocyte number (broken line)

these data, intravascular coagulation was suggested, and treatment with PID was started on Sept. 22nd, 1962 guided by the Thrombotest method (Owren) During treatment for three weeks the Thrombotest values ranged between 6 and 12 %. Gradually she regained movement of the right leg On Oct. 8th, she developed signs of a deep venous thrombosis in the left leg (Thrombotest 12 %) and she was admitted to Department IX, Ullevål City Hospital for further examinations.

On admission, she was cachectic. The liver was enlarged, and her left leg swollen and tender Haemoglobin had dropped to 9.5 g % Alkaline phosphatase 50 Bodansky units. The P P value was 96 % and plasma fibrinogen 0 178 g/100 ml The number of thrombocytes was 50,000/mm³ In addition, small amounts of fibrin in plasma were observed (see below) Again, there was no evidence of fibrinolysis.

These observations seemed to indicate that treatment with PID was ineffective in its ability to inhibit intravascular coagulation. Therefore, PID treatment was discontinued on Oct. 11th, and for the next six days intravenous injections of heparin (45 000 units daily) were given. During this treatment, plasma fibrinogen and thrombocytes rised 3—4-fold (fig 1) and fibrin could no longer be demonstrated in plasma. The tendency to bleed (haematomata and oozing following venous punctures) noted on PID treatment disappeared. The bleeding time was also normal during heparin treatment.

In order to see if the effect was only coincidental, heparin was discontinued on Oct. 17th. As is evident from fig 1 however both fibrinogen and thrombocyte numbers fell very rapidly to pretreatment values, and fibrin reappeared in plasma. At the same time, her condition deteriorated rapidly In the afternoon, Oct. 18th she was comatose with irregular respiration. Treatment with heparin was resumed but she died the following day

The post mortem examination revealed a cerebral haemorrhage with a large, partly cystic haematoma in the left hemisphere. The pancreas had a 4 × 3 cm tumour in the body Microscopical examination showed a typical pancreatic adenocarcinoma. The liver showed multiple metastatic nodules. The surrounding liver tissue was intensely congested. Microscopical examination revealed extensive thrombus-formation in portal veins and also widespread fibrin deposition in sinusoids surrounding the metastatic nodules. The spleen had several small areas of infarction, as well as the left kidney and there was a small pulmonary embolus. Examination of the heart revealed verrucous endocarditis of the aortic cusps with deposition of platelets and fibrin but no leucocytes and no bacteria.

Demonstration of preformed fibrin in plasma

No precipitation occurred if citrated plasma from the patient was incubated at 37 C overnight. On cooling (ice water or

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From the Medical Department IX (Head: K. A. Aas, M. D.)
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A Follow-up Study of 330 Patients with Myocardial Infarction, with Particular Reference to Development of Heart Failure

By

Jan H. Solem, Ivar Helle and Wilfred Jorgensen

I course of the past two to three decades there have appeared a number of studies on the prognosis in patients with myocardial infarction with special reference to recurrent infarction and the length of survival time, particularly in relation to anticoagulant therapy. The incidence of heart failure seems to have been given much less attention, and only little information has been obtained concerning the factors which can influence the development of heart failure.

Material

The follow-up study comprises all males aged 30 to 64 years who were discharged from Departments VIII and IX, Ullevaal Hospital, Oslo, after having survived a first attack of myocardial infarction during the period 1956—1959. The observation period started at discharge and the clinical follow-up examinations were performed in the course of 1961. However all patients have been followed with respect to survival until 31 December 1961. The total number of patients discharged was 340. By the end of 1961 72 patients had died. As our data on 8 of the surviving and 2 of the dead patients are quite unsatisfactory the study comprises 330 patients of whom 260 were alive and 70 had died.

The vast majority, i.e. 249 of the surviving and 62 of the dead patients, had received anticoagulant therapy in the hospital in connection with the acute infarction. Continuous long-term therapy was upheld in 185 patients, while such therapy had been stopped after an average of one year of therapy in 133 patients.

The examinations were made at the hospital usually in the out-patient clinic, sometimes during hospitalization. As to the deceased patients, information was obtained from hospital records or from the physician in charge in 47 cases, while in 23 cases it was possible to get information from the family only.

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Results

Table I shows th distribution of patients over the fou years and the age distribution on discharge. As many as 239 of the 330 patients were between 50 to 64 years of age.

Heparin had an excellent symptomatic effect on the hypercoagulable state, whereas treatment with PID was in effective.

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The examinations were made at the hospital usually in the out-patient clinic, sometimes during hospitalization. As to the deceased patients, information was obtained from hospital records or from the physician in charge in 47 cases, while in 23 cases it was possible to get information from the family only.

Results

Table I shows the distribution of patients over the four years and the age distribution on discharge. As many as 259 of the 330 patients were between 50 to 64 years of age.

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Table IV Relationship between incidence of heart failure and age

Age group	Total	Without heart failure			With heart failure			% of total with heart failure
		Total	Survived	Dead	Total	Survived	Dead	
30—49	71	66	55	11	5	3	2	7.0
50—59	174	141	118	23	33	18	15	19.0
60—64	83	63	51	12	20	13	7	24.1
Total	328	270	224	46	58	34	24	17.7

who survived the study period (table III) Reliable data on angina pectoris were not available for the patients who died.

The incidence of heart failure due to infarction is apparent from table IV The diagnosis of heart failure was based on the presence of one or more of the following criteria paroxysmal nocturnal dyspnea, a diagnosed pulmonary edema or manifest peripheral edema. The number of patients was 328, as two patients were excluded because the heart failure was present prior to the infarction. In these 328 patients the diagnosis of heart failure following infarction was made in 58 cases. The table shows that the tendency to develop heart failure following myocardial infarction increases markedly with advancing age.

Left heart failure only was diagnosed in 23 of 58 patients.

Table V shows the significance of some other factors with reference to development of heart failure. Recurrent infarction and hypertension wer more frequent in patients with heart failure.

In 8 of the 34 living patients the heart failure was not diagnosed prior to our follow-up examination, despite the fact that all 8 were on continuous long term anticoagulant therapy

In 9 of the 34 living patients manifest heart failure had developed already during the first 6 months of the observation period. Reliable data concerning the first symptoms and signs of frank heart failure were not available for the patients who died. It is, however apparent that the incidence of manifest failure does not necessary increase with the duration of the observation period. Among the 63 patients who were discharged in 1956 and for whom the observation period thus had been at least 5 years, heart failure was diagnosed in 16, or 19.3 %. The age distribution in 1956 corresponds well with that in the total material.

Table V Relationship between incidence of heart failure and other factors of significance

	Total	Without heart failure	With heart failure
No. of patients	328	270	58
Recurrent infarction (not leading to death)	25	7	18
Definite diastolic hypertension	38	13	25
Rheumatic heart disease	3	1	2
Other heart disease.	4	2	2
Chronic pulmonary disease	42	34	8
Diabetes mellitus	14	14	0

Table I Patients in 5 year age-groups calendar year of discharge and deaths prior to 1st Jan. 1962

Age group	Total		Dead prior to 1st Jan. 1962	
	No.	%	No.	% of total
30–34	2	0.6	–	–
35–39	8	2.4	3	–
40–44	20	6.1	3	15.0
45–49	41	12.4	7	17.1
50–54	72	21.8	17	23.6
55–59	104	31.5	21	20.2
60–64	83	25.2	19	22.9
Total	330	100	70	21.2
Year of discharge				
1956	83	25.2	20	24.1
1957	85	25.7	22	25.9
1958	93	28.2	16	17.2
1959	69	20.9	12	17.4
Total	330	100	70	21.2

Table II. Patients deceased prior to 1st Jan. 1962 by cause of death

	No. of pat.
Certain recurrent infarction (autopsy)	17
Probable recurrent infarction	17
Sudden death	23
Heart failure	5
Pulmonary emboli	1
Cerebral hemorrhage	1
Other diseases	6
Total	70

Table III Presence of angina pectoris

I. Among the 330 patients before admission

Angina pectoris	Total		Dead prior to 1st Jan. 1962	
	No.	%	No.	% of total
For > 6 months	102	30.9	32	31.4
For < 6 months	43	13.0	8	18.6
Not present	185	56.1	30	16.2
Total	330	100	70	21.2

II. Among the 260 surviving patients during the observation period

	Angina pectoris before admission			
	Present		Not present	
	No.	%	No.	%
Previous angina pectoris definitely improved	23	21.9	–	–
Previous angina pectoris apparently unimproved	25	23.8	–	–
Previous angina pectoris not improved	57	54.3	–	–
Angina pectoris developed during the observation period	–	–	79	51.0
No angina pectoris at any time	–	–	76	49.0
Total	105	100	155	100

Table II shows the causes of death and reveals that the majority of patients who die after myocardial infarction suffer a "coronary" death.

In association with the follow up study the patients were investigated as to angina pectoris prior to and following recovery from the myocardial infarction. With reference to previous angina pectoris the criterion used was the presence of anginal pain for more than four weeks prior to the infarction. The incidence of angina pectoris following an acute infarction was only estimated in the patients

during the first month after the gradual discontinuation of the therapy had been initiated. In most cases discontinuation was completed in the course of two to three weeks.

Angina pectoris prior to the initial infarction was observed in 43.9 of the patients, an incidence which corresponds well with that commonly reported in the literature (about 50 %) Two thirds of these patients had had angina pectoris for more than 6 months before the occurrence of the infarction The incidence of angina pectoris following recovery from the acut infarction was recorded in the 260 surviving patients. It appeared that real improvement of the angina occurred n 21.9 of the cases. An apparent improvement was recorded in 23 8 of the cases When these patients, however were more thoroughly questioned, the amelioration could be explai ed by the f

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(4) found that 13 % had developed congestive failure, in all cases associated with enlarged heart. He further found severe dyspnea in 15 8 % of the patients without hypertension and in 26 4 in the hypertensive group Palmer found that the lapse of time between the onset of the attack and the appearance of failure was 1 6 years on the average and that a clear majority of the patients with heart failure had hypertension We found recurrent infarction and hypertension relatively more frequently in the group with heart failure than n the others.

The overweight of patients with chronic pulmonary disease in the non failure group (table V) may partly be due to the fact that we were anxious not to overdiagnose heart failure in patients with chronic pulmonary diseases. Since our material mostly comprises patients in the older

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Table VI. Working ability at time of clinical re-examination

	Same work as before infarction	Started lighter work	Not at work	Total
Without heart failure	73 (32.6 %)	108 (48.2%)	43 (19.2%)	224 (100%)
With heart failure	6 (16.7%)	10 (27.8%)	20 (55.5%)	36 (100%)
Total	79 (30.4%)	118 (45.4%)	63 (24.2%)	260 (100%)

Major reason for not working at time of re-examination

Heart failure	13
Angina pectoris	19
Heart failure and angina pectoris	5
Recurrent heart infarction	1
Cardiac neurosis	2
Other causes	23
Total	63

Table VII. The cardiac volume estimated radiologically at re-examination in 255 patients

Cardiac vol. (ml/sqm body surface area)	Total	With heart failure	
		No	% of total
≦490	182	11	6.0
500–540	27	5	18.5
≧550	46	17	37.0
Total	255	33	12.9

One of the 34 patients with heart failure was among the 5 living patients who could not be followed up with radiological heart examination.

The 260 patients alive at the termination of the observation period were examined with regard to their working ability (table VI). Slightly more than 75 per cent of the patients were at work after recovery from the acute infarction. Heart failure and/or angina pectoris were the main causes of inability to resume work. In about one third of the patients who were not at work (recorded under "other causes") the chief reason was that they had reached retiring age.

Radiological examination of the heart was carried out in 255 patients at the follow up study. Table VII shows the relationship between heart failure and the size of the heart. As normal we have regarded cardiac volumes less than 500 ml per square meter body surface area (that is relative volume). Border line volumes mean relative volumes between 500 and 540 ml and definitely increased volumes 550 ml or more. These criteria have been used according to the method of radiological examination and volume calculation used by Amundsen (1). He found that the cardiac volume in men is normally ≦ 490 ml per square metre body surface area and definitely increased at 550 ml or above.

Discussion

Of the 330 patients in our follow up study, 25 had recurrent infarction with a non lethal course during the observation period. Of these 14 received anticoagulant therapy at the time of the recurrence and 11 did not. In a total of 133 patients only one case of definite recurrent infarction (with a non-lethal course) was observed in direct connection with discontinuation of the anticoagulant therapy, that is

during the first month after the gradual discontinuation of the therapy had been initiated. In most cases discontinuation was completed in the course of two to three weeks.

Angina pectoris prior to the initial infarction was observed in 43.9 % of the patients, an incidence which corresponds well with that commonly reported in the literature (about 50 %). Two thirds of these patients had had angina pectoris for more than 6 months before the occurrence of the infarction. The incidence of angina pectoris following recovery from the acute infarction was recorded in the 260 surviving patients. It appeared that a real improvement of the angina occurred in 21.9 % of the cases. An apparent improvement was recorded in 23.8 of the cases When these patients, however were more thoroughly questioned the amelioration could be explained by the fact that the patients had modified their way of life according to their pain limit On the other hand 31 % of the patients with no previous angina pectoris had developed this symptom during the observation period. This last percentage corresponds well with the few publications on this particular subject, for instance Palmer (4) with a value of 46 %.

Of 260 patients about three fourths were at work at the time of our follow-up This is an encouraging result, considering that about two thirds of the 260 patients felt their working capacity reduced due to dyspnea, angina pectoris or weakness.

As to the incidence of heart failure following myocardial infarction, relatively little attention seems to have been given t this problem. In a study on 212 patients observed for an average period of 4 9 years after having survived a first attack of myocardial infarction Palmer (4) found that 13 / had developed congestive failure in all cases associated with enlarged heart. He further found severe dyspnea in 15.8 of the patients without hypertension and in 26 4 / in the hypertensive group. Palmer found that the lapse of time between the onset of the attack and the appearance of failure was 1 6 years on the average, and that a clear majority of the patients with heart failure had hypertension We found recurrent infarction and hypertension relatively more frequently in the group with heart failure than in the others.

The overweight of patients with chronic pulmonary disease in the non-failure group (table V) may partly be due to the fact that we were anxious not to over diagnose heart failure in patients with chronic pulmonary diseases. Since our material mostly comprises patients in the older age groups in whom chronic bronchitis and pulmonary emphysema is common t has been often difficult to determine the relative shares of heart failure and lung disease in the production of shortness of breath. There were no cases of failure among the patients with diabetes mellitus, a phenomenon which so far remains unexplained. It may be suggested that diabetics with myocardial infarction die a coronary death" before they develop failure, but a more detailed analysis f our patients with diabetes mellitus does not confirm this explanation.

Of deciding significance for development of heart failure following infarction was th age of the patients (table IV) This important factor is not mentioned by Palmer

In patients with a normal heart size we found symptoms of failure in only 6 % of the cases. This bears out a finding of practical importance previously pointed

out by Amundsen (1) i e. that a normal sized heart is seldom associated with congestive failure. On the other hand 63 % of our patients with cardiac enlargement had no heart failure according to our strict diagnostic criteria. If we segregate the 30 patients with a relative heart volume of above 600 ml we find that 13 that is nearly half of them, had no clinical symptoms of heart failure. The present follow up study thus reveals that patients who have had myocardial infarction may have enlargement of the heart without manifest symptoms of failure. In a follow up study of 412 patients with myocardial infarction Master and Jaffe (3) found a complete functional recovery in 154 of whom 25 had cardiac enlargement and/or marked electrocardiographic changes. By a closer examination of our material we found that of 47 patients who at discharge had an enlarged heart (estimated radiologically) 38 developed heart failure during the observation period.

It is evident that the prognosis in patients who survive an attack of myocardial infarction is poorer for patients who develop heart failure than for patients without failure. In their follow up study of 162 patients with myocardial infarction Bland and White (2) found that of 44 patients who developed heart failure none was alive 10 years after the acute attack, while 56 % of 55 patients with complete recovery survived the 10-year period.

It must be pointed out that in about 25 % of the 34 surviving patients with heart failure, failure was not diagnosed before our follow up All of these patients received long term anticoagulant treatment and should thus, at least theoretically have been under regular medical supervision. *It should therefore be stressed* that closer attention to heart failure is advisable in the treatment of patients having had myocardial infarction.

Summary

The present communication comprises a follow up study of 330 male patients between 30 and 64 years of age who were discharged from Ullevaal Hospital during 1956—1959 after having survived a first attack of myocardial infarction.

Heart failure subsequent to the attack occurred in 7 % of the cases in the youngest age group, in 19 % in the medium group and in 24 1 % in the eldest group Recurrent myocardial infarction and hypertension were found more frequently among the patients who had developed heart failure than among those who recovered without failure. In 25 % of the cases with heart failure, the failure was not diagnosed before the follow up study The authors therefore stress that closer attention with respect to heart failure is recommended in patients who survive an attack of myocardial infarction.

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Development of Diabetes Mellitus in a Patient Suffering from Addison's Disease

By

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The coexistence of diabetes mellitus and Addison's disease is very rare. Since the observation of Ogle in 1886 (14) about 80 cases have been reported (2, 5, 9, 11, 17). From Hungary Barna (1) has observed a case in 1956. In his case the two diseases were diagnosed at about the same time. The simultaneous occurrence of the two syndromes presents many problems, it seemed therefore worth while to describe in detail our case which is rendered still more interesting by the fact that diabetes developed in a patient suffering for years from hypocorticalism, whereas in over two thirds of the cases described in the literature diabetes was diagnosed initially and adrenocortical insufficiency occurred only later.

Case report

A 22-year-old man was admitted to the Endocrine Unit of our Department in August 1962. His history extends as far back as 8 years. His complaints then were weakness, giddiness, loss of weight and malaise. His skin had a brownish pigmentation, his blood pressure was 90/80 mm Hg, serum sodium 127, serum potassium 4,6, serum chloride 95 mEq/l, 17 ketosteroids 6,1 mg/24 hr. Basal metabolic rate −3 %. Blood glucose curve: 82—141—102—68 mg/100 ml. The diagnosis of hypadrenia was established. He was given a diet rich in sodium and vitamins, treated with desoxycorticosterone-acetate and for years had no further complaints. It should be emphasized that he never received cortisone or prednisolone preparations and that glucose loading tests were performed repeatedly, always with normal results.

On admission to our Department in Aug. 1962 he told us that for about 10 days he had not felt well, was weak and felt dizzy. He did not mention any polyuria or polydipsia. In his family history neither diabetes nor Addison disease had occurred.

He weighed 64 kg being 170 cm high. His weight had not changed appreciably in the course of the last weeks.

At physical examination his skin had a diffuse brown colour, on the mucous membrane of the mouth a little brownish pigmentation could be observed. His blood pressure was 110/70 sitting and 100/70 mm Hg standing.

The more important laboratory findings were: specific gravity of the urine 1,020. Nylander' reaction in the urine positive, acetone could not be demonstrated. Fasting blood sugar 246 mg/100 ml, daily output of urine 1,000—2,000 ml, sugar excretion be

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tween 20 to 40 g. Serum potassium 6.0, serum sodium 124, serum chloride 89 mEq/l. Blood urea nitrogen 45 mg/100 ml. ECG: sinus rhythm, in leads I—II and V_{1-4} high sharp T waves which were considered to be signs of hyperpotassemia. After the potassium level became normal, the ECG alterations ceased. Tuberculin test (Mantoux) at a dilution of 1 : 100 000 positive. Neutral total 17-ketosteroid excretion 3.5—5.9 mg/24 hours (Holtorff-Koch method according to modification of Faredin et al. (7)); total 17-hydroxycorticosteroid excretion 1.52—2.80 mg/24 hours (Glenn-Nelson method modified according to Faredin (8)); total pregnandiol excretion 1.30—2.30 mg/24 hours (with the method of Sommerville et al. (15)).

The patient was ill. On the basis of the laboratory findings in addition to the previously confirmed Addison's disease we also diagnosed diabetes mellitus. Because of the greater sensitivity to insulin which could be expected owing to the presence of Addison's disease we attempted to improve his condition only by the application of dietetic and infusion treatment. A diet containing 200 g carbohydrate was given and hypertonic NaCl infusions were applied.

As his general condition had improved significantly the endocrinological investigations were continued. The plasma hydrocortisone level (by means of the paper chromatographic method of Gláz et al. (10)) was unmeasurably low. After an insulin load (0.1 IU normal insulin per kg body weight intravenously) the blood sugar curve was 101—74—54—64—74—91—101—95 mg/100 ml (thus it seems that enhanced insulin sensitivity could not be demonstrated). During the insulin loading test the plasma hydrocortisone level did not increase. A glucose loading test (50 g glucose orally) resulted in 137—230—282—339—?89 mg/100 ml, a typical diabetic curve being obtained. The plasma insulin-like activity (measured by Vallance-Owen's rat hemidiaphragm technique (16) modified according to Bretán and Kammerer (3)) ranged between 10^{-4}—10^{-4} mg/ml which can be considered normal. After the application of a carbutamide load (3 g orally) the blood glucose fell within 2 hours from 101 mg/100 ml to 83 mg/100 ml and after 4 hours to 76 mg/100 ml, thus carbutamide sensitivity was present. At the end of the loading test the insulin-like activity of the plasma showed a moderate increase. Owing to hypersensitivity ACTH loads could not be applied, following prednisolone administration the urinary sugar excretion and the blood sugar values increased considerably. After a water load a significant water retention could be observed. Administration of desoxycorticosterone-acetate did not influence the urinary sugar excretion and the blood sugar level.

On discharge from our Department his fasting blood glucose level was 130 mg/100 ml, his urinary sugar excretion 0—?0 g/24 hours.

Discussion

In patients suffering from Addison's disease carbohydrate metabolism is usually impaired. The blood glucose level is usually normal or lower and if oral glucose loads are applied the blood sugar curves are generally flat. The enhanced response to insulin of patients suffering from Addison's disease is well known. Even if only small doses of insulin are given severe hypoglycemic reactions may frequently develop (12). This is due to the reduced glucocorticoid secretion. If hypadrenia develops in patients in whom diabetes is already present the diabetic condition usually improves, as in the case of the observations made in the animal experiments of Long and Lukens (13). Thus, it may be easily understood why diabetes mellitus can only rarely be found in patients suffering from Addison's disease but the fact of its occurrence at all has not yet been elucidated.

In our patient diabetes mellitus developed after more than 8 years of hypocorticalism. Considering that he never received cortisone or prednisolone the possibility of a steroid diabetes may be ruled out. In spite of his youth his diabetes turned out not to be the so-called "brittle" insulin dependent type. Neither abnormally enhanced insulin sensitivity nor

pathologically low plasma-insulin-like activity could be demonstrated. His diabetes could be stabilized without administration of insulin by means of an adequate diet. The decrease of the blood glucose level and rise in the insulin-like activity following a carbutamide load also suggest that insulin production has not ceased in this patient. In our opinion the most important conclusion which can be drawn from this case is the establishment that even if the functional capacity of one of the most important factors of the contra-insular system, the adrenal cortex, decreases diabetes mellitus may develop with practically normal insulin level. Of course, the question arises as to which factors took over the insulin antagonistic role of the adrenal cortex? To this we cannot give a definite reply this problem can only be elucidated by further experiments.

Owing to the coexistence of the two diseases certain therapeutic problems also arise. Taking the antagonism between the insular pparatus and the adrenal cortex into account the treatment of Addison disease should be restricted as far as possible to administration of mineralocorticoids and enhanced intake of sodium, respectively If the application of glucocorticoids becomes necessary insulin treatment can usually not be avoided (4 6) On the other hand, if owing to the seriousness of the diabetic state insulin must be given, owing to the enhanced insulin sensitivity even if only small doses are used severe hypoglycemic reactions must be expected. In such cases cortisone or prednisolone administration exerts stabilizing effect (9 17) In our case an adequate diet and treatment with desoxycorticosterone-acetate seemed for the present sufficient to control the two diseases.

Summary

The case of a man aged 22 years is reported. After the patient had been suffering for 8 years from Addison's disease he developed diabetes mellitus. Considering that these two diseases are very rarely associated his case history is described in detail. Enhanced insulin sensitivity could not be observed in the patient, and insulin-like activity of the plasma was also normal. The observations suggest that diabetes mellitus may also develop when the functional capacity of the adrenal cortex is reduced and the insulin level is normal. The therapeutic problems of the associated occurrence of the two diseases are also discussed.

Acknowledgement

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Histamine Studies in a Case of Zollinger-Ellison's Syndrome

A Preliminary Report

By

G. Dotevall, S. E. Lindell and H. Westling

The Zollinger Ellison's syndrome is now thought to consist of gastric hypersecretion and a persistent tendency to peptic ulceration associated with a tumour or hyperplasia of the pancreas, or more rarely in other endocrine glands. The pancreatic tumour has been shown to contain a secretagogue, which is very similar to gastrin (5) and it seems fair to assume, that this is the cause of the hyperactivity of the parietal cells of the stomach.

In the view of the possible connection between histamine and gastric secretion it appeared of interest to study some aspects of histamine metabolism in a patient with Zollinger Ellison's syndrome.

Case report

A previously healthy woman, aged 31 developed symptoms of peptic ulcer during the spring of 1960. X-ray of the stomach showed coarse mucosal contour of "gastritis" type. In Dec. 1960, during her third pregnancy she was taken acutely ill and caesarean section was performed. During this operation blood was found in the intestines, and probable duodenal ulcer was palpated. During the spring of 1961 ulcer symptoms recurred. A radiological examination showed a coarse and thickened mucosa in both stomach and duodenum. The duodenal cap was slightly deformed but no ulceration could be seen. After treatment with antacids the patient was free from symptoms until July 1961 Her ulcer pains now recurred and were more intensive, radiating to the back. Fatty diarrhoea was also frequent. Radiological examination showed the same picture as previously Various examinations pertaining to the gastrointestinal function were performed and the details will be published elsewhere (1) Gastric secretion was studied repeatedly by the method of Kay (8) The results of these studies are shown in fig 1

Fig. 1 shows that before operation the patient had copious basal secretion of hydrochloric acid, which was only slightly raised by maximal histamine stimulation. Atropine caused a reduction by about 50 % of the basal secretion.

At laparotomy on Nov 28, 1961 a tumour with diameter of roughly 1 inch was found in the head of the pancreas. The head of the pancreas was removed together with the duodenum and the lower part of the antrum,

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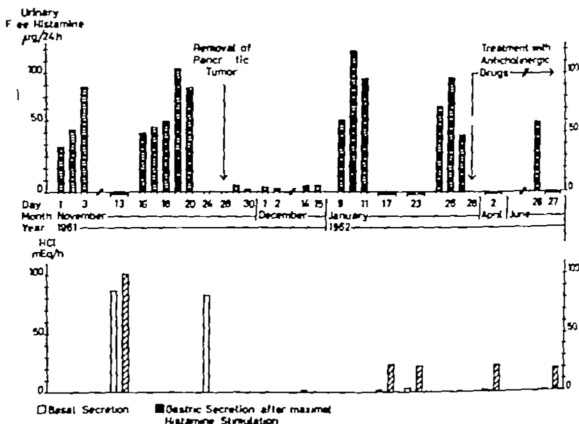


Fig. 1. Urinary free histamine in μg base per 24 hrs and gastric acid secretion in mEq/l before and after operation. Upper range of normal urinary free histamine in women is 30 μg/24 hrs.

the main part of the stomach being left intact. The antrum and the proximal opening of the jejunum were anastomosed end-to-end and the common duct and pancreas inplanted to a U-shaped jejunal loop according to Cattell, the two jejunal branches being anastomosed to one another. The tumour showed a histological picture similar to that in previously published cases (13). Part of the tumour was sent to Professor R. A. Gregory, at the Department of Physiology, University of Liverpool, who found a considerable gastrin-like activity in an extract of the tumour as in previously published cases (5, 6).

After the operation the basal gastric secretion became normal. The response to a maximal histamine stimulation was also normal: the increase in HCl output after histamine was about the same as before operation.

Histamine metabolism. The 24-hour urinary excretion of free histamine was measured by bioassay on guinea-pig ileum after extraction according to Dunér and Pernow (3). Fig. 1 shows the observations made on the urinary histamine. Before operation the daily excretion of free histamine was significantly higher than normal. After removal of the pancreatic tumour the urinary excretion of histamine fell to values that were actually lower than normal.

The agent in the urine that caused the contraction of the guinea-pig ileum was specifically antagonized by mepyramine (9) and therefore considered to be biologically identical with histamine. It was found that urine collected during the period of low histamine excretion immediately after operation did not contain substances which disturbed the extraction of histamine from the urine or its assay on the ileum. Careful examination of the urinary sediment and repeated bacteriological culture of the urine did not reveal any urinary infection during the entire observation period.

Discussion

The reason for the high urinary excretion of free histamine in this case is not known. It seemed possible that the patient might have a decreased capacity to metabolize histamine. This hypothesis was tested by a study of the metabolism of injected C[14] labelled histamine according to the method of Schayer and Cooper (10). The patient's capacity to metabolize the labelled histamine was entirely normal, about 2 % of the injected amount appearing in the urine in unchanged form. 79 % of the injected C[14] was excreted in 12 hours of these 2 % was histamine, 3 % was methylhistamine, 66 % was methylimidazoleacetic acid and 23 % was imidazoleacetic acid. Thus the increased excretion of histamine should be due to either increased liberation and/or formation of histamine.

It is well known that gastric juice contains histamine regardless of the mechanism of stimulation and it seemed possible that the urinary histamine might come from the stomach. The fact that urinary histamine excretion fell after removal of the tumour fits well with this idea, especially since there was also a pronounced decrease in the secretion of hydrochloric acid. It must be noted however that within 6 weeks of surgery the urinary histamine increased to the preoperative level without any concomitant increase in the gastric secretion of HCl. It thus seems unlikely that the urinary excretion of histamine in this case was directly related to the production of HCl in the stomach.

In January 1962 the patient had a peptic ulcer in the jejunum. It is possible that the increased excretion of histamine was related to this ulceration. However other cases of recurrent duodenal ulcer did not show an increased excretion of histamine (2).

It is known that rapidly growing tissues have a high capacity to form histamine (7) and the urinary histamine might thus have been formed in the tumour. The return of an increased excretion of histamine 6 weeks after surgery could then be due to recurrence of the tumour. The histidine decarboxylase activity of the excised tumour tissue measured with Schayer's technique was rather low but still higher than that in the surrounding parts of the pancreas which gives some support to this hypothesis.

Finally it should be mentioned that Smith (11, 12) found that gastrin released histamine from various tissues in the cat. This finding has not been confirmed in dogs (4) but the possibility must be considered that in our patient gastrin produced by the tumour tissue released histamine, part of which was excreted in the urine.

The case is reported in order to focus the attention on the possibility of an alteration in histamine metabolism in a patient with the Zollinger Ellison's syndrome, and it is suggested that patients having or supposed to have this syndrome, should have their urinary excretion of histamine examined.

Summary

A case of Zollinger Ellison's syndrome with a high urinary excretion of histamine is described.

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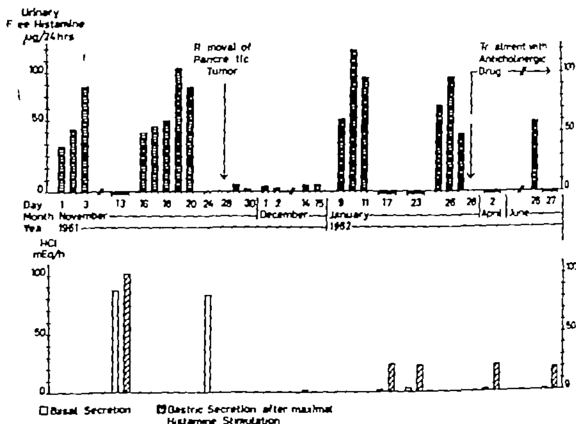


Fig 1 Urinary free histamine in µg base per 24 hrs and gastric acid secretion in mEq/l before and after operation. Upper range of normal urinary free histamine in women is 50 µg/24 hrs.

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Chronic Relapsing Pancreatitis

I. Clinical Manifestations of Acute Attacks and Possible-aetiologic Factors

By

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Clinical manifestations of chronic relapsing pancreatitis still frequently escape correct diagnostic evaluation though more than fifteen years have elapsed since its classical description by Comfort et al. (8) W feel therefore that it will be useful to report on the clinical manifestation of chronic relapsing pancreatitis in 75 patients observed in 1958—1961 including 28 who had already been followed up in our institute during the previous period. In the present paper we shall deal with the symptomatology of acute exacerbations and possible aetiologic factors. The results of auxiliary examinations during the quiescent period will be included in part II of the present communication.

Definition

The term of chronic relapsing pancreatitis implies an affection of the external pancreatic secretion characterised by relapses in the form of sublethal attacks of acute pancreatitis. The summation of these attacks and their morphological sequelae are the basis of clinical manifestation of chronic relapsing pancreatitis. The course of the disease is progressive and is characterised by the alternation of acute episodes and quiescent intervals. During the acute stage the clinical symptomatology is dominated by pain and biochemical changes caused by increased passage of pancreatic enzymes into interstinal spaces and blood and by the development of necrotic foci in the pancreatic tissue. During the quiescent period the disease manifests itself by functional external pancreatic insufficiency and also in a certain percentage of patients, by internal pancreatic insufficiency the occurrence of pancreatic calcifications and pseudocysts. In very rare instances chronic relapsing pancreatitis is not associated with pain.

Repeated exacerbations increase the extent of fibrotic changes and functional insufficiency as well as the other clinical manifestations. Therefore during the advanced stage of the disease sometimes the caracteristic alternation of quiescent periods and attacks ceases, and the patients suffer from permanent complaints which increase in a paroxysmal fashion.

Method

Chronic relapsing pancreatitis was diagnosed in all patients from repeated recurrences of acute episodes of which at least two were

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Table III. Probable genesis of chronic relapsing pancreatitis in 75 patients

Cholecystolithiasis	Choledocho-lithiasis	Parapapillary diverticulum	Alcoholism	Factor not detected
36 (48.0%)	14 (18.7%)	7 (9.3%)	3 (4%)	29 (38.7%)

Table IV. Frequency, character and location of pain in 75 patients with chronic relapsing pancreatitis

Incidence	Character			Location			
	Pressure	Cramping	Colicky	Right hypo-chondrium	Epi-gastrium	Left hypo-chondrium	Back
75 (100%)	9 (12%)	59 (78.6%)	7 (9.4%)	6 (8%)	44 (58.6%)	14 (18.7%)	11 (14.7%)

Table V. Direction of extension of pain during acute stage in 75 patients with chronic relapsing pancreatitis

T the left	T the right	T the back	Lower part of the abdomen	T left and back	Combined directions of extension	
					T left and left lower part of chest	T right and right lower part of chest
38 (50.7%)	13 (17.3%)	25 (33.3%)	2 (6.7%)	17 (22.7%)	12 (16%)	6 (8%)

Alcoholism as factor contributing to the exacerbation of acute episodes in these patients is extremely rare (4).

Among our patients 47.9 % suffered from cholecystolithiasis and, moreover 18.6 % from choledocholithiasis (table III). We cannot draw any conclusions on the frequency of stenosing papillitis since in the patients who were operated on radiomanometry was never performed. The finding obtained by intubation is not considered reliable (14). Therefore the finding of six cases of papillitis in 35 operated patients does not give an accurate picture of its incidence and its possible participation in the development of chronic relapsing pancreatitis. A duodenal diverticulum in the neighbourhood of the papilla of Vater was found in seven patients (9.4 %).

I all patients of this group, pain was the leading symptom of the acute stage. The pain is characterized by its location, direction of its extension and duration (table IV V VI). As regards the remaining symptoms (table VII) nausea was most frequent in our patients (54.6 %), flatulence (41.3 %) vomiting (38.6 %) and diarrhoea (22.6 %). From total of 17 patients (22.7 %) who suffered from diarrhoea 12 (16) reported that the stools were light-coloured while the colour of the urine was normal.

As regards other signs, loss of weight and an elevated concentration of pancreatic enzymes in the blood were most frequent (tables VII, VIII). The blood amylase concentration was investigated in all 75 patients, the lipase concentration only in 38 patients (50.8 %)

Table I Sex, average age and body weight of 75 patients suffering from chronic relapsing pancreatitis

Sex		Average age	Familial incidence	Obesity	Normal weight	Under weight	Weight not given
♀	♂						
35 (46.7%)	40 (53.3%)	48.9 —	0 —	21 (28%)	36 (48%)	12 (16%)	6 (8%)

Table II Probable cause of development of acute attack in 75 patients with chronic relapsing pancreatitis

Cholelithiatic attack	Dietary error	Alcoholism	Physical strain	Stress	Cause not detected
5 (6.6%)	35 (46.6%)	3 (4%)	6 (8%)	3 (4%)	23 (30.7%)

diagnosed — in 61 patients by ourselves with clinical and laboratory methods, and in 14 patients at other departments In 65 patients evidence of impaired pancreatic function was provided by means of the secretin test. In 35 patients, including seven where the secretin test was not performed, the diagnosis was confirmed by the finding of fatty necroses or fibrotic enlargement of the pancreas on operation for cholelithiasis. Amylase was estimated by our modification of Teller's method (9) estimations by Wohlgemuth's method (33) being carried out in other departments. We consider as elevated values those above 170 Somogyi units for the modified method of Teller and those above 256 units for Wohlgemuth's method. Lipase was estimated by Frič's modification of Rade recht's method (10) elevated values are those above 3 1 mg %. Serum calcium was estimated by titration with complexon III using fluorexon as indicator (25) — normal values 8—11 6 mg % bilirubin by Jendrassik—Grof's method (17) — normal values up to 1 mg % alkaline phosphate by King-Armstrong's method (19) — normal values up to 10 units. The blood sugar level was assessed simultaneously with amylase by Teller's method the range of normal values is 60—110 mg

The secretin test was carried out by separate withdrawal of gastric and duodenal juice by means of two Emhorn tubes one was introduced into the lower portion of the stomach, the second to the duodenojejunal flexure. The location of the tubes was always checked by X-ray The concentration of bicarbonates was estimated manometrically by van Slyke's method, and the total secretion determined during 60 min. following i.v administration of 80 units "Sekretin Spofa" or 75 units "Sekretinum Vitrum" Using this technique, we found in the control group a maximum bicarbonate concentration somewhat above 90 mEq/l and a secretion of 1.9 ml/kg (12)

As is apparent from table I in this group of patients with chronic relapsing pancreatitis men were involved more frequently than women though not as frequently as in some other series of patients. The average age of patients was 48.9 years the youngest patient was 21 years old, the oldest 67 A familial incidence of chronic relapsing pancreatitis was not observed in this group, nor did we find a more frequent incidence of the disease in obese patients. The body-weight was taken as normal unless its value in kg diverged by more than ± 10 from the height expressed in cm minus 100.

The most frequent cause of exacerbation of acute episodes was a dietary error in the form of a rich food, frequently eaten moreover in excessive amounts (table II) Only in five patients (6 6 %) was the development of the acute episode associated with a biliary colic. All these patients suffered at the same time from cholecysto- and choledocholithiasis.

Table III. Probable genesis of chronic relapsing pancreatitis in 75 patients

Cholecystolithiasis	Choledocholithiasis	Parapapillary diverticulum	Alcoholism	Factor not detected
34 (45.9%)	14 (18.7%)	7 (9.3%)	3 (4%)	29 (38.7%)

Table IV. Frequency, character and location of pain in 75 patients with chronic relapsing pancreatitis

Incidence	Character			Location			
	Pressure	Cramping	Colicky	Right hypochondrium	Epigastrium	Left hypochondrium	Back
75 (100%)	9 (12%)	59 (78.6%)	7 (9.4%)	6 (8%)	44 (58.6%)	14 (18.7%)	11 (14.7%)

Table V. Direction of extension of pain during acute stage in 75 patients with chronic relapsing pancreatitis

To the left	To the right	To the back	Lower part of the abdomen	To left and back	Combined directions of extension	
					To left and left lower part of chest	To right and right lower part of chest
38 (50.7%)	13 (17.3%)	25 (33.3%)	2 (6.7%)	17 (22.7%)	12 (16%)	6 (8%)

Alcoholism as a factor contributing to the exacerbation of acute episodes in these patients is extremely rare (4 %).

Among our patients 47.9 % suffered from cholecystolithiasis and, moreover, 18.6 % from choledocholithiasis (table III). We cannot draw any conclusions on the frequency of stenosing papillitis since in the patients who were operated on radiomanometry was never performed. The finding obtained by intubation is not considered reliable (14). Therefore the finding of six cases of papillitis in 35 operated patients does not give us an accurate picture of its incidence and its possible participation in the development of chronic relapsing pancreatitis. A duodenal diverticulum in the neighbourhood of the papilla of Vater was found in seven patients (9.4 %).

In all patients of this group, pain was the leading symptom of the acute stage. The pain is characterized by its location, direction of its extension and duration (table IV, V, VI). As regards the remaining symptoms (table VII), nausea was most frequent in our patients (54.6 %), flatulence (41.3 %), vomiting (38.6 %) and diarrhoea (22.6 %). From a total of 17 patients (22.7 %) who suffered from diarrhoea 12 (16 %) reported that the stools were light-coloured while the colour of the urine was normal.

As regards other signs, loss of weight and an elevated concentration of pancreatic enzymes in the blood were most frequent (tables VII, VIII). The blood amylase concentration was investigated in all 75 patients, the lipase concentration only in 38 patients (50.8 %)

Table I Sex average age and body weight of 75 patients suffering from chronic relapsing pancreatitis

Sex		Average age	Familial incidence	Obesity	Normal weight	Under-weight	Weight not given
♀	♂						
35 (46.7%)	40 (53.3%)	48.9 —	0 —	21 (28%)	36 (48%)	12 (16%)	6 (8%)

Table II Probable cause of development of acute attack in 75 patients with chronic relapsing pancreatitis

Cholelithiatic attack	Dietary error	Alcoholism	Physical strain	Stress	Cause not detected
5 (6.6%)	35 (46.6%)	3 (4%)	6 (8%)	3 (4%)	23 (30.7%)

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The most frequent cause of exacerbation of acute episodes was a dietary error in the form of a rich food, frequently eaten moreover in excessive amounts (table II). Only in five patients (6.6 %) was the development of the acute episode associated with a biliary colic. All these patients suffered at the same time from cholecysto- and choledocholithiasis.

Discussion

The clinical manifestation of chronic relapsing pancreatitis was overlooked for a long time. Though it was known that in some patients with cholelithiasis the pain has an atypical location and extension, this was not ascribed to chronic pancreatitis. Only Katch (18) drew attention to some symptoms and signs of chronic pancreatitis, particularly some typical features of pancreatogenic pain, and thereby also elucidated the cause of the contralateral location and direction of extension of the pain. The occurrence of relapses of acute pancreatitis was also known. Only Comfort et al. (5) however demonstrated that in most of these instances a special type of chronic pancreatitis, chronic relapsing pancreatitis, is involved. According to our present experience chronic relapsing pancreatitis is the clinically most pronounced type of chronic pancreatitis (12) So far however no definite conclusions can be made on its incidence. According to Hess (14) chronic relapsing pancreatitis accounts for 37 % and according to our experience for 79.5 % of the total cases of chronic pancreatitis (12) It must be emphasised that Hess' statistics are surgical and ours medical.

In 1956 one of us (H.) assembled from the literature 363 patients suffering from chronic relapsing pancreatitis and supplemented them by 73 of our own patients (12) From the total number of 430 patients 225 (58.5 %) were men. Also in this second group with chronic relapsing pancreatitis men predominate 1 4 1 Generally it is found that men suffer more frequently from the condition than women (3 7 16, 20 32) One of the assumed reasons is more frequent alcoholism in men (3 7 11 32) It was assumed that alcohol influences the external secretion of the pancreas by the sequelae of its direct action on the gastric and duodenal mucosa, via the blood stream. Dreiling et al. (6) however provided evidence that injections of 200—600 ml 5 % ethyl alcohol do not affect the external secretion of the pancreas, either in controls or in people suffering from chronic relapsing pancreatitis. This effect of alcohol therefore does not participate in the development of chronic relapsing pancreatitis.

To 24 patients with chronic relapsing pancreatitis, 6—15 months after the last acute episode, we administered by intraduodenal tube 2 ml of 40 % isotonic ethyl alcohol/kg up to a maximum dose of 190 ml. The dose was selected according to the results of Menguy's experiments (24) In 75 % of the patients we found an increased amylase or lipase activity in blood — by more than 100 % approaching or exceeding the normal upper level. In the control group the enzyme activity remained always within the range of normal values. The maximum rise of amylase was 80.5 % of lipase 85.5 % (13) We explain this effect of 40 % alcohol by its local effect on the duodenal mucosa and on the papilla of Vater These changes together with structural changes, changes in the configuration of the efferent pancreatic ducts and changes in the secretory capacity of acinal cells probably determine in patients with chronic relapsing pancreatitis whether after intraduodenal administration of alcohol increased amounts of pancreatic enzymes pass into the blood stream. The decisive factor in this effect of alcohol is probably its concentration.

Ethyl alcohol in the gastrointestinal tract influences the external pancreatic secretion in two ways. 1 Alcohol administered into any part of the digestive

Table VI Duration of pain during acute stage in 75 patients with chronic relapsing pancreatitis

24 hrs	2 days	3 days	5 days	> 5 days
10 (13.3%)	35 (46.6%)	15 (20%)	8 (10%)	7 (9.3%)

Table VII. Frequency of other symptoms during acute exacerbation in 75 patients with chronic relapsing pancreatitis

Flatulence	Diarrhoea	Constipation	Light coloured faeces	Nausea	Vomiting	Loss of weight	Shock	Fever	Jaundice	Impaired glyco-regulation
31 (41.3%)	17 (22.7%)	7 (9.3%)	12 (16%)	41 (54 7%)	29 (38.7%)	75 (100%)	17 (22.6%)	15 (20%)	16 (21.3%)	10 (13.3%)

Table VIII. Results of biochemical examinations in 75 patients with chronic relapsing pancreatitis during acute stage

	Amylase	Lipase	Calcium	Alkaline phosphatase	Bilirubin	Blood sugar	Leuko-cytes
No. of pat.	75 (100%)	38 (50.6%)	31 (54.6%)	42 (56%)	42 (56%)	38 (50.6%)	22 (29.3%)
Normal values	1 (1 4%)	9 (23.7%)	19 (61.9%)	22 (52.4%)	26 (61.9%)	28 (73.7%)	0 (0%)
Elevated values	74 (98.6%)	29 (76.3%)	0 (0%)	20 (47 6%)	16 (38.1%)	10 (26.3%)	22 (100%)
Reduced values	0 (0%)	0 (0%)	12 (38.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

(table VIII) In one patient (1 4 %) a normal amylase level was found repeatedly during two relapses the lipase level was found repeatedly during two relapses the lipase level was, however elevated in both instances. The blood sample from this patient was taken 24 hours after the excerbation of the relapse. In the remaining patients — 98 6 % — the amylase level was markedly elevated. The lipase concentration was elevated in 76 3 % of the total of 38 patients examined The serum calcium, alkaline phosphatase, bilirubin and the blood sugar level were not investigated in all patients and during every relapse. Calcium was assessed in 54 6 %, alkaline phosphatase and bilirubin in 56 %, and the blood sugar level in 37.3 %. A reduced calcium level was found in 38.1 % and an increased blood sugar level in 26.3 % of the patients where these examinations were made. In all 22 patients (29.3 %) where the differential blood count was examined during the exacerbation of the relapse, leukocytosis was revealed.

calised the pain most frequently in the midepigastrium, less frequently in the left epigastrium or the back, and only in rare instances in the right epigastrium. The patients described the pain most frequently as boring, or cramping usually very intense. Only in rare instances was the pain mild, pressure-like or on the contrary colicky In addition to the epigastric location and its intensity also the left direction of extension is also characteristic for the pain. When the pain is in the right epigastrium, it is referred through to the left, to the midepigastrium and sometimes to the left epigastrium. When the pain is localised in the mid epigastrium or left epigastrium, it extends along the left costal arch. Relatively frequently the pain radiated in our patients to the back, very rarely to the right. In two patients the pain spread into the lower portion of the abdomen. The pain caused by the necrotic focus of pancreatic tissue often extends simultaneously in several directions — most frequently to the left along the left costal arch either to the back or to the left lower half of the chest, less frequently to the right and the right lower half of the chest. The radiation of pain to the left is very valuable from the diagnostic point of view Every pain localised in the upper half of the abdomen spreading to the left raises in the first place the suspicion of pancreatogenic origin.

In addition to the direction of extension, the pain caused by the formation of a necrotic focus of pancreatic tissue is characterized by its duration. In 65 patients (86.7 %) the pain persisted for more than 24 hours in 35 patients (46.7 %) for two days, in 15 (20 %) for three days, in eight (10.7 %) for five days and in seven (9.3 %) for more than five days. In ten patients (13.3 %) the pain persisted for less than 24 hours. The duration of pain its predominant location in the epigastrium and its extension to the left are typical features of pain caused by the formation of a necrotic focus of pancreatic tissue.

The rise of the pancreatic enzyme in the blood is the main sign of the acute stage. In 98.6 % of the patients in this group during the acute stage the amylase level rose and in 76.3 % also the lipase level. The lipase levels were, however estimated in only 50.6 % of all patients. It is therefore probable that if lipase had been estimated in all patients, the percentage would be even higher There fore we cannot take the observation of a raised lipase level in one of our patients where the amylase level remained unchanged as evidence of the actual frequency of this discrepancy in the behaviour of pancreatic enzymes in the blood during the exacerbation of the acute episode.

Various authors (2, 12, 32) have pointed to differences, in the frequency and time of maximum changes, between the lipase and amylase blood levels in patients with acute pancreatitis or its relapses. The rise of lipase in the interstitium, lymph and blood is much more important for the organism than the rise of amylase. It can be assumed that an effective system of inhibitors begins to act, as during a rise of proteolytic enzymes. This probably renders it difficult to get direct evidence of a raised lipase activity in the blood of some patients with acute pancreatitis or its relapses even if there is an increased passage into the blood stream.

The elevated serum concentration of pancreatic enzymes has the same diagnostic value for the acute stage as has functional insufficiency of the external

system including the isolated stomach and intestinal loop (34 35) stimulates the humoral mechanism of gastric juice secretion with a high HCl and a small pepsin content. The higher the acidity of gastric juice the more it stimulates secretin formation in the duodenum. In experimental animals after total gastrectomy this stimulation does not occur (31) 2 Alcohol in the duodenum causes spasms of the sphincter of Oddi and when administered in larger amounts and higher concentrations it causes oedema of the duodenal mucosa and papilla of Vater It is assumed that this blocks the flow of pancreatic juice and thus leads to a raised pressure in the efferent ducts (24)

In Czechoslovakia beer of a concentration not exceeding 5 % is drunk more frequently than concentrated alcoholic beverages. This is probably one reason why only rarely did we encounter increased over consumption of alcohol in our patients with chronic relapsing pancreatitis.

McPhedran and Lucas did not find pancreatic changes on histological examination of rats fed for six months 15 / alcohol alone or with a diet. In countries where large amounts of concentrated alcoholic beverages are consumed alcohol is an important factor in the development of chronic relapsing pancreatitis (3 7 11) Since despite the low percentage of alcoholics in our group of patients with chronic relapsing pancreatitis, in 1956 as well as in the present group men predominated a greater abuse of alcohol probably is not the only reason why the disease occurs more frequently in men

The incidence of cholelithiasis and choledocholithiasis in this group of patients with chronic relapsing pancreatitis — 47.9 % — does not exceed the figures reported in the literature (17) It is, however questionable whether in all these patients the affection of the efferent biliary pathways can be considered primary and whether in some of these patients two aetiological independent diseases might be involved

In our patients with chronic relapsing pancreatitis the incidence of parapapillary diverticula is relatively high. As early as in 1919 Åkerlund (1) drew attention to the fact that patients with a parapapillary diverticulum are predisposed to acute and chronic pancreatitis. The participation can be ascribed both to the spread of the inflammation from the diverticulum to the efferent pancreatic ducts and the subsequent influence on the pressure in these ducts, and to the direct penetration of the diverticulum into the head of the pancreas (24) All patients in whom we discovered during the period between 1958 and 1961 a parapapillary diverticulum suffered also from chronic relapsing pancreatitis. In view of the small number of patients with a parapapillary diverticulum no conclusions can be drawn from this fact. Nevertheless it emphasizes that when a parapapillary diverticulum is found the possibility of simultaneous chronic relapsing pancreatitis must be considered and if the latter is diagnosed the parapapillary diverticulum may be considered in agreement with Svartz and Sjoberg (29) the possible cause of the development of chronic relapsing pancreatitis

The main clinical symptom of the acute episode of chronic relapsing pancreatitis is pain and the main sign is an elevated concentration of pancreatic enzymes in serum.

All 75 patients in this group reported pain as their main complaint. They lo-

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Table IX. Duration of disease in 75 patients with chronic relapsing pancreatitis

6 months	1 year	2 yrs	3 yrs	4 yrs	5 yrs	> 5 yrs
5 (6.6%)	10 (13.3%)	12 (16%)	8 (10.7%)	5 (6.6%)	5 (6.6%)	30 (40%)

secretion ascertained by the secretin test, for the diagnosis of the quiescent stage. It must, however be taken into account that in patients with a perforated peptic ulcer (26) with obstruction of the small intestine (22) and acute obstruction of the afferent loop (15) the pancreatic enzymes in blood may be likewise elevated Therefore the elevated level must be evaluated always in relation to the clinical picture as a whole. The rise of enzymes is sometimes very transient. Therefore when the findings are normal allowance must also be made for the time which elapsed between the exacerbation of the acute stage and the withdrawal of the blood specimen The more this period exceeds 48 hours the less weight can be given to a normal enzymatic finding particularly for amylase, as evidence against the existence of a necrotic focus of pancreatic tissue. Then further symptoms and signs must be evaluated particularly the character location, direction of extension and duration of pain the serum calcium level the blood sugar level, and the white cell count in subsequent relapses the need should be anticipated for examination of blood for the pancreatic enzymes as soon as possible after the onset.

Apart from the rise of pancreatic enzymes in blood we found, in all patients in whom during the onset of the acute attack a differential blood count was made, an increased number of leukocytes. Leukocytosis is a non-specific symptom, as in other inflammatory abdominal conditions. The calcium, blood sugar, bilirubin and alkaline phosphatase level were not investigated in all patients (table IX) A reduced calcium level is found relatively frequently during the development of necrotic foci in pancreatic tissue (8 21) We found it in 38.7 % of a total of 31 patients in whom this examination was made. In view of the fact that chronic pancreatitis develops in some patients with hyperparathyroidism, the calcium level must be examined in every patient during the acute as well as the quiescent stage of chronic relapsing pancreatitis. The decrease of the calcium level is important for the diagnosis of the acute stage. The finding of normal values during the acute stage calls for examination during the quiescent stage, in order to ascertain whether the normal serum level during the acute stage was not merely a decrease of the raised level to normal values. The degree of decline of the serum calcium level, as for the rise of the leukocyte count, has also a prognostic value. The greater the decline in the calcium level and the greater the rise of the white blood count, the more serious the prognosis of the acute stage. In our experience no prognostic conclusions can be drawn from the degree of the rise in amylase and lipase.

It is important to assess whether during the acute stage of chronic relapsing pancreatitis there is no rise in the concentration of alkaline phosphatase and bilirubin in serum The cause of the rise is most frequently cholangitis caused by

incomplete obstruction of the common bile duct, less frequently complete obstruction of the choledochus or a pancreatic concrement which got stuck in the papilla of Vater The sequelae of repeated acute attacks can cause such an enlargement of the head of the pancreas that it causes either permanent incomplete obstruction which aggravates the changes caused by the acute attack, or transient incomplete or complete obstruction confined to the acute stage and accompanied by clinical manifestations of obstruction. With the regression of the acute attack sometimes the excessive pressure likewise recedes. Thus chronic cholangitis developing in conjunction with attacks of acute exacerbating pancreatitis is a serious complication and an absolute indication for elimination of the excessive pressure by surgical operation. The excessive pressure may also, t a certain stage, manifest itself biochemically Therefore the estimation of the bilirubin and alkaline phosphatase serum levels during the acute stage of chronic relapsing pancreatitis is ery important. It must be, however taken into account that the patient with chronic relapsing pancreatitis suffers sometimes from choledocholithiasis with concomitant cholecystolithiasis, so that the excessive pressure is not always of pancreatogenic origin The differentiation can be made by intra enous or sometimes only by peroperative cholangiography Only in 16 (21.3) of the patients in this group jaundice did develop in the course of the acut ttack. These manifestations of hronic relapsing pancreatitis in patients of our group will be the subject of another communication.

In the course of the development of chronic relapsing pancreatitis, a certain percentage of patients ho develop functional insufficiency of the internal secretion of the pancreas (3 , 12, 30) The disorder is usually at first transient and manifests itself only during periods of acute exacerbations. If the inflammatory changes affect also a considerable portion of the endocrine tissue, diabetes develops. Ten patients of this group (13.3 %) developed hyperglycaemia and glycosuria during the acute exacerbation. In 6 (8 %) of the patients the disorder receded after regression of the acute episode. It manifested itself, however by a pathological blood sugar curve during the quiescent stage. In four (5.3 %) the disorder persisted permanently

The presence of impaired glyco-regulation during the acute stage is very valuable from the diagnostic point of view Every sudden attack during which a raised blood sugar level or glycosuria can be found raises the suspicion of pancreatogenic origin. The impaired glyco-regulation is due to a more extensive affection of the insular tissue (30) Therefore it is found more frequently in patients with chronic relapsing pancreatitis than in those suffering from the first attack of acute pancreatitis (3 12, 28)

Chronic relapsing pancreatitis is a chronic disease. In 48 patients (64 %) it has already persisted for more than two years, including 35 patients (46.7 %) where it has persisted for five or more years. The term chronic thus describes not only the existence of chronic inflammatory changes of the exocrine part of the pancreas which developed as a *result* of relapses of the acute attacks, but also the chronic course of the disease. The clinical manifestation of the acute stage of chronic relapsing pancreatitis is *typical* and even if it is not fully developed it cannot escape attention, if it is considered in the differential diagnosis.

Table IX Duration of disease in 75 patients with chronic relapsing pancreatitis

6 months	1 year	2 yrs	3 yrs	4 yrs	5 yrs	> 5 yrs
5 (6.6%)	10 (13.3%)	12 (16%)	8 (10.7%)	5 (6.6%)	5 (6.6%)	30 (40%)

secretion ascertained by the secretin test, for the diagnosis of the quiescent stage. It must, however be taken into account that in patients with a perforated peptic ulcer (26) with obstruction of the small intestine (22) and acute obstruction of the afferent loop (15) the pancreatic enzymes in blood may be likewise elevated. Therefore the elevated level must be evaluated always in relation to the clinical picture as a whole. The rise of enzymes is sometimes very transient. Therefore when the findings are normal, allowance must also be made for the time which elapsed between the exacerbation of the acute stage and the withdrawal of the blood specimen The more this period exceeds 48 hours, the less weight can be given to a normal enzymatic finding particularly for amylase, as evidence against the existence of a necrotic focus of pancreatic tissue. Then further symptoms and signs must be evaluated particularly the character location direction of extension and duration of pain, the serum calcium level the blood sugar level and the white cell count in subsequent relapses the need should be anticipated for examination of blood for the pancreatic enzymes as soon as possible after the onset.

Apart from the rise of pancreatic enzymes in blood we found, in all patients in whom during the onset of the acute attack a differential blood count was made, an increased number of leukocytes. Leukocytosis is a non specific symptom, as in other inflammatory abdominal conditions. The calcium blood sugar bilirubin and alkaline phosphatase level were not investigated in all patients (table IX) A reduced calcium level is found relatively frequently during the development of necrotic foci in pancreatic tissue (8 21) We found it in 38.7 % of a total of 31 patients in whom this examination was made. In view of the fact that chronic pancreatitis develops in some patients with hyperparathyroidism, the calcium level must be examined in every patient during the acute as well as the quiescent stage of chronic relapsing pancreatitis. The decrease of the calcium level is important for the diagnosis of the acute stage. The finding of normal values during the acute stage calls for examination during the quiescent stage, in order to ascertain whether the normal serum level during the acute stage was not merely a decrease of the raised level to normal values. The degree of decline of the serum calcium level, as for the rise of the leukocyte count, has also a prognostic value. The greater the decline in the calcium level and the greater the rise of the white blood count, the more serious the prognosis of the acute stage. In our experience no prognostic conclusions can be drawn from the degree of the rise in amylase and lipase.

It is important to assess whether during the acute stage of chronic relapsing pancreatitis there is no rise in the concentration of alkaline phosphatase and bilirubin in serum. The cause of the rise is most frequently cholangitis caused by

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Summary

The authors submit an account of the manifestation of the acute stage of chronic relapsing pancreatitis and possible aetiologic factors in 75 patients in 35 of the patients the diagnosis was confirmed at operation.

Concomitant cholecystolithiasis was present in 47 9 % of the patients, choledocholithiasis in 18 6 % A parapapillary diverticulum was found in 9 3 % alcoholism in 4 % of the patients. The probable cause of the development of the disease could not be traced in 38.7 %

The most frequent probable cause of the development of the acute attack was a dietary error (46 4 %) In five patients (6 6 %) the exacerbation of the acute stage occurred in conjunction with a biliary colic. All these patients suffered from concomitant cholecysto- and choledocholithiasis The participation of alcoholism in the development of relapses was established only in 3 patients (4 %)

The leading symptom of the acute episode is pain — of certain character location direction of extension and duration As regards other symptoms the most frequent complaint was flatulence and light-coloured stools. The other so-called minor symptoms are of small diagnostic value.

As regards objective signs the most valuable is the raised level of pancreatic enzymes in the blood stream. It is essential always to examine also the blood calcium blood sugar bilirubin and alkaline phosphate levels since chronic relapsing pancreatitis may cause excessive pressure in the extrahepatic bile ducts and impairment of the internal and external secretory components of the pancreas. The clinical manifestation of the acute stage of chronic relapsing pancreatitis is typical even when not fully developed and cannot escape attention if it is considered in the differential diagnosis.

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From the Institute of Hygiene, University of Oslo, Oslo, Norway

Studies on Hemoglobin Values in Norway

II. The Effect of Supplementary Intake of Ascorbic Acid and Iron on the Hemoglobin Level of School-children and Men

By

Haakon Natvig, Tor Bjerkedal and Øyvind Jonassen

In an earlier study the hemoglobin level was found to vary to only a slight extent from one part of Norway to another (13). The study did not, however, include observations from the northern part of Norway, but other investigators claim to have found particularly low hemoglobin levels for adults and children living north of the Arctic Circle (5, 8, 15, 20).

Marked seasonal variations in the hemoglobin level in these areas have also been reported. Several investigators (1, 7, 10) have agreed with the view expressed by Finsen (6) that the hemoglobin level is influenced by the intensity of sunlight; this, he maintains, accounts for the low average hemoglobin level among populations inhabiting the far north, and for the seasonal variation with the lowest values in the dark winter. Other workers, however, have found the lowest values in spring and summer (2, 11, 12, 17), a feature they have ascribed to the influence of nutritional factors. It is argued that in spring and early summer there is a relative shortage of certain types of food, particularly meat and vegetables, in the far northern areas. As a consequence, vitamin C and iron, both of which are important for maintaining a normal hemoglobin level (3, 18, 19), may be insufficient or suboptimal at this season, so that the lowest hemoglobin values occur in the early summer.

If this explanation is correct it would be expected that a daily supplementary intake of vitamin C and iron would raise the allegedly low average hemoglobin levels in northern Norway to those prevailing in the southern part of the country and, moreover, eliminate the seasonal variation. To ascertain whether this does in fact occur, a study of the effect of a twelve-month supplementary intake of ascorbic acid and iron on the hemoglobin level was carried out on a population group in northern Norway.

The present paper deals with the effect of the supplementary supply *per se* on the hemoglobin levels. In a subsequent article the observations will be considered as they relate to the problem of seasonal variations in hemoglobin level.

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Table II. Average number of tablets consumed per person per month in the various groups

Age (yrs)	Placebo	Ascorbic acid	Iron	Ascorbic acid + iron
Men				
30–39	19.7	20.5	20.0	20.8
50–59	21.3	20.8	20.2	20.8
Boys				
10–13	18.9	19.5	19.1	19.1
Girls				
10–13	19.1	19.7	19.6	19.6

Table III. Number of subjects reporting discomfort related to tablet taking

Age (yrs)	Total	Placebo	Ascorbic acid	Iron	Ascorbic acid + iron
Men					
30–39	2	—	—	—	2
50–59	9	3	1	3	2
Boys					
10–13	0	—	—	—	—
Girls					
10–13	1	—	1	—	—
Total	12	3	2	3	4

the four groups, these differed in size at the end of the study. Table I shows the original and final composition of the groups, and the numbers lost and excluded.

Supply of ascorbic acid and iron

Each participant was given, as far as possible, one of four different tablets on each working day throughout one year. The four types of dragees were identical in size, colour and appearance, both external and internal. One type contained 100 mg ascorbic acid, another 30 mg iron in the form of ferro-fumarate, and the third 100 mg ascorbic acid and 30 mg iron; the fourth was a placebo. Analyses of the tablets (F. Rakh, Nyegaard & Co, A/S) on 29th Jan., 20th June 1960 and 7th Feb. 1961 showed that there was no change in their composition during the period of the study. The tablets were supplied in bottles of 100, marked A, B, C, or D on different coloured labels. Neither the participants nor the other persons concerned with the study knew the code until the data had been analysed.

In accordance with instructions the foremen at A/S Sydvaranger and the teachers at the elementary school on every working day gave each of the participants one tablet, the type of which had been determined by drawing lots. When the tablet had been taken the foreman or teacher entered a cross on the subject's monthly record card. By this means an accurate record was kept of the number of tablets consumed by each participant throughout the period of the experiment.

The average number of tablets consumed per month was satisfactory and there was no essential difference in this respect between the test groups (table II). Since approximately the same number of subjects in the placebo groups as in the other groups stated that they did not tolerate the tablets, it would seem that none of the tablets had any marked side effects (table III).

As a result of holidays and other days off from work, illness, absence on business, military service and civil defence exercises, it was impossible to obtain blood samples for hemoglobin determinations from all the subjects every month. The average number of hemoglobin determinations for both men and school-children was, however, about the same in all four test groups (table IV).

Blood specimens and method for hemoglobin determination

The hemoglobin determinations were performed monthly throughout the period of the experiment — that is from 1st Feb., 1960 until 31th Jan., 1961. The blood specimens were taken each time in the same order arranged in such a way as to avoid any differences between the groups as a result of systematic errors of measurement. All the blood

The tablets were prepared for the investigation by Nyegaard & Co, A/S, Oslo.

Table I Composition of the material

Age (yrs)	Initial no.	Lost or rejected	No. on which analysis is based				
			Total	Placebo	Ascorbic acid	Iron	Ascorbic acid + iron
Men							
30—39	202	33	169	44	36	44	45
50—59	110	19	91	23	25	23	20
Boys							
10—13	92	6	86	22	21	23	20
Girls							
10—13	76	3	73	19	18	19	17
Total	480	61	419	108	100	109	102

Material and methods

The study was performed at Kirkenes, an industrial town of about 10 000 inhabitants on the 70° parallel. The conditions here were highly suitable, since the mining company A/S Sydvaranger which is the most important industry in the region, with 1,500 employees, has a well organized medical service, in which one of the present authors (O J) is the works physician.

The material of the study consisted of two groups of subjects, one of men and the other of school-children. No women were included in the study because of the variability of the hemoglobin level due to menstruation, pregnancy and child birth.

The adult group comprised 312 men, all of them employed at A/S Sydvaranger Participation was restricted to two age groups, 30—39 and 50—59 years, with 202 in the former and 110 in the latter All had been recorded as being in sound health at the previous examination by the works physician there had been no illnesses or conditions that might be suspected of affecting the hemoglobin level the subjects felt fit and were willing to take part in the study

The groups of school-children consisted of 92 girls and 76 boys aged 10—13 years, all of them attending Kirkenes Elementary School. This age class was chosen so as to avoid complications introduced by the marked rise in hemoglobin level at puberty while at the same time having children old enough to permit blood samples to be taken once a month. All the children had been recorded as being in sound health at the previous examination by the Schools Medical Officer

The men and the children were assigned to four groups which were to receive ascorbic acid iron, ascorbic acid and iron or placebo tablets. The division was made in such a way that the dispersion of the hemoglobin values and the mean, as determined in January 1960 were as nearly as possible the same for the four groups. To ensure that the groups were comparable also with respect to other factors on which the hemoglobin content of the blood might depend, a subsequent adjustment was performed by rendering the groups uniform with regard to weight, ESR, place of work, position annual income and income tax. The groups to which supplementary ascorbic acid and iron were to be taken were decided by drawing lots. The participants were asked not to take pills, tablets or tonics containing vitamin C or iron and if they did so to report it.

Owing to migration, illness during the period of the study and other such factors, about 17 % of the men and 5 % of the school-children were lost to the investigation. Since this waste was not the same for each of

Table V. Mean Hb values (g/100 ml blood) for 30—39 year group of men

Date	Placebo		Ascorbic acid		Iron		Ascorbic acid + Iron	
	No.	Mean	No.	Mean	No.	Mean	No.	Mean
1960								
18—25/1	44	16,1	36	16.0	44	16.0	43	16.0
19—27/2	41	15,7	36	15.7	38	15.5	44	15.6
18—26/3	39	15,7	36	15.4	41	15.3	38	15.3
23—30/4	41	15.5	35	15.2	39	15.0	44	15.1
20—28/5	41	15.2	30	15.2	40	15.1	42	15.2
18—22/6	36	15.4	31	15.0	36	15.0	36	14.9
15—23/7	31	14.2	24	14.2	27	14.3	27	14.2
19—27/8	38	15.4	30	15.1	36	15.5	38	15.1
23—30/9	41	15.5	31	15.3	38	15.5	39	15.1
21—29/10	38	15.6	30	15,1	37	15.5	39	15.1
18—26/11	39	15.2	34	15.4	40	15.4	39	15.1
16—22/12	40	15.2	32	15.2	40	15.2	39	15.2
1961								
20—28/1	40	15.4	34	15.2	39	15.2	38	15.1
All observations	505	15.4	419	15.2	495	15.5	508	15.2

Table VI. Mean Hb values (g/100 ml blood) for 50—59 year group of men

Date	Placebo		Ascorbic acid		Iron		Ascorbic acid + Iron	
	No.	Mean	No.	Mean	No.	Mean	No.	Mean
1960								
18—25/1	23	15.4	25	15.8	23	15.7	20	15.4
19—27/2	22	15.3	23	15.7	25	15.8	18	15.4
18—26/3	23	15.1	24	15.0	21	15.3	20	15.0
23—30/4	22	14.8	23	15.0	19	14.8	15	14.7
20—28/5	23	14.9	25	15.1	22	15.0	14	14.7
18—22/6	19	14.6	23	15.8	20	15.1	18	14.9
15—23/7	18	13.9	18	14.1	12	14.2	13	14.0
19—27/8	21	14.9	21	15.3	21	15.3	19	15.1
23—30/9	20	15.1	23	15.0	22	14.8	19	15.1
21—29/10	22	15.2	23	15.1	23	15.4	18	15.1
18—26/11	23	14.9	25	15.2	21	15.4	18	15.3
16—22/12	19	14.9	24	15.2	20	15.2	19	15.0
1961								
20—28/1	23	14.9	24	15.3	22	15.1	18	14.9
All observations	278	14.9	301	15.2	269	15.2	229	15.0

Table IV Average number of Hb determinations per person in the various groups. The men were scheduled for 13 Hb determinations, and the school-children for 12 determinations during the 12 months of the study

Age (yrs)	Placebo	Ascorbic acid	Iron	Ascorbic acid + iron
Men				
30—39	11.6	11.6	11 4	11.5
50—59	12.2	12 0	11.6	11.5
Boys				
10—13	11 1	11.6	11 4	11 4
Girls				
10—13	11 0	11.6	10.8	11 4

samples and the readings were taken by a nurse who was specially engaged and trained for this work. She was unaware of the results of previous tests on the individual subjects, for after each month s tests the list of results was sent to the Institute of Hygiene in Oslo together with all the monthly record cards showing the individual consumption of tablets.

All the blood tests were performed at the place of work or at the school. The blood tests were taken from the fingertip with Sera Sharp Blood Lancets (Propper Manufacturing Co. Inc., N Y) After removing the first few drops with cotton wool, 0.20 ml of blood was withdrawn in a dry adjusted pipette and immediately mixed with 3 5 ml of 0 1 solution of sodium carbonate.

The hemoglobin determination was performed by means of a Linson Junior apparatus (AB Lars Ljungberg & Co., Stockholm.) 2—4 hours after taking the sample. With this apparatus which functions on the oxyhemoglobin principle, readings can be taken to an accuracy of one per cent. The method is very suitable for large-scale studies it is extremely simple and reproducible, and accurate results can be obtained after a short period of training The apparatus, with the cuvettes, and the conversion factor were checked at the Central Laboratory Ullevål Hospital against oxyhemoglobin and cyanmethemoglobin in a Beckman DU apparatus. For the conversion a millimolar extinction coefficient of 11.5 was used and the molecular weight of hemoglobin was taken as 16.520.

The hemoglobin values in this study cannot be compared directly with the values given in Study I (13) since different methods and extinction coefficients were used. However an approximate comparison can be made by reducing the values for Study I by 6% (9 16)

Determination of the ascorbic acid level in plasma, the serum-iron and the total iron-binding capacity of blood

To obtain an impression of the extent to which the supplementary intake of ascorbic acid and iron had affected the ascorbic acid content of plasma, the serum-iron content, and the total iron-binding capacity of the blood, blood samples for determination of these values were drawn from a random sample of men in the 30–39 year group immediately after the last blood specimen had been taken for hemoglobin determination in January 1961 After precipitation of the plasma with metaphosphoric acid, the ascorbic acid was estimated by titration with 2 6-di-chlorophenolindophenol sodium as an indicator

The serum-iron was assayed by Ramsay's method and the total iron-binding capacity of blood (TIBC) by the method given by Peters et al. The blood samples for these determinations were taken in special tubes and examined at the Central Laboratory Ullevål Hospital.

Results

A Hemoglobin determinations

The mean hemoglobin values for the four test groups of men were fairly uniform for any particular month with no systematic inter-group differences (tables V and VI) The group means for the twelve-month period were also closely similar the highest and lowest means for the 30—39 year group differing by only 0.2 g or 1 5 per cent, and those for the 50—59 year group by only 0.3 g or 2.0 per cent. If the two age groups are combined, the following means are obtained

Ascorbic acid 15.2 g, iron 15.2 g, ascorbic acid + iron 15 3 g placebo 15.2g

Table V. Mean Hb values (g/100 ml blood) for 30—39 year group of men

Date	Placebo		Ascorbic acid		Iron		Ascorbic acid + iron	
	No.	Mean	No.	Mean	No.	Mean	No.	Mean
1960								
18—25/1	44	16.1	36	16.0	44	16.0	43	16.0
19—27/2	41	15.7	36	15.7	38	15.5	44	15.6
18—26/3	39	15.7	36	15.4	41	15.3	38	15.5
23—30/4	41	15.3	35	15.2	39	15.0	44	15.1
20—28/5	41	15.2	30	15.2	40	15.1	42	15.2
18—22/6	36	15.4	31	15.0	36	15.0	36	14.9
15—23/7	31	14.2	24	14.2	27	14.3	27	14.2
19—27/8	38	15.4	30	15.1	36	15.3	38	15.1
23—30/9	41	15.3	31	15.3	38	15.3	39	15.1
21—29/10	38	15.6	30	15.1	37	15.5	29	15.1
18—26/11	39	15.2	34	15.4	40	15.4	39	15.1
16—22/12	40	15.2	32	15.2	40	15.2	38	15.2
1961								
20—28/1	40	15.4	34	15.2	39	15.2	38	15.1
All observations	509	15.4	419	15.2	495	15.3	408	15.2

Table VI. Mean Hb values (g/100 ml blood) for 50—59 year group of men

Date	Placebo		Ascorbic acid		Iron		Ascorbic acid + iron	
	No.	Mean	No.	Mean	No.	Mean	No.	Mean
1960								
18—25/1	23	15.4	25	15.8	23	15.7	20	15.4
19—27/2	22	15.3	23	15.7	23	15.6	18	15.4
18—26/3	23	15.1	24	15.0	21	15.3	20	15.0
23—30/4	22	14.8	23	15.0	19	14.8	15	14.7
20—28/5	23	14.9	25	15.1	22	15.0	14	14.7
18—22/6	19	14.6	23	15.0	20	15.1	18	14.9
15—23/7	18	13.9	18	14.1	12	14.2	13	14.0
19—27/8	21	14.9	21	15.3	21	15.3	19	15.1
23—30/9	20	15.1	23	15.0	22	14.8	19	15.1
21—29/10	22	15.2	23	15.1	23	15.4	18	15.1
18—26/11	23	14.9	25	15.2	21	15.4	18	15.3
16—22/12	19	14.9	24	15.2	20	15.2	19	15.0
1961								
20—28/1	23	14.9	24	15.3	22	15.1	18	14.9
All observations	278	14.9	301	15.2	260	15.2	229	15.0

Table IV Average number of Hb determinations per person in the various groups. The men were scheduled for 13 Hb determinations, and the school-children for 12 determinations during the 12 months of the study

Age (yrs)	Placebo	Ascorbic acid	Iron	Ascorbic acid + iron
Men				
30–39	11 6	11 6	11 4	11.5
50–59	12.2	12.0	11.6	11.5
Boys				
10–13	11 1	11 6	11 4	11 4
Girls				
10–13	11 0	11.6	10.8	11 4

samples and the readings were taken by a nurse who was specially engaged and trained for this work. She was unaware of the results of previous tests on the individual subjects, for after each month s tests the list of results was sent to the Institute of Hygiene in Oslo, together with all the monthly record cards showing the individual consumption of tablets.

All the blood tests were performed at the place of work or at the school. The blood tests were taken from the fingertip with Sera Sharp Blood Lancets (Propper Manufacturing Co. Inc., N Y) After removing the first few drops with cotton wool, 0.20 ml of blood was withdrawn in a dry adjusted pipette and immediately mixed with 3.5 ml of 0.1 solution of sodium carbonate.

The hemoglobin determination was performed by means of a Linson Junior apparatus (AB Lars Ljungberg & Co Stockholm.) 2—4 hours after taking the sample With this apparatus which functions on the oxyhemoglobin principle, readings can be taken to an accuracy of one per cent. The method is very suitable for large-scale studies It is extremely simple and reproducible, and accurate results can be obtained after a short period of training The apparatus, with the cuvettes, and the conversion factor were checked at the Central Laboratory Ullevål Hospital against oxyhemoglobin and cyanmethemoglobin in a Beckman DU apparatus. For the conversion a millimolar extinction coefficient of 11.5 was used and the molecular weight of hemoglobin was taken as 16,520

The hemoglobin values in this study cannot be compared directly with the values given in Study I (13) since different methods and extinction coefficients were used. However an approximate comparison can be made by reducing the values for Study I by 6% (9 16).

Determination of the ascorbic acid level in plasma, the serum-iron and the total iron-binding capacity of blood

To obtain an impression of the extent to which the supplementary intake of ascorbic acid and iron had affected the ascorbic acid content of plasma, the serum iron content, and the total iron binding capacity of the blood, blood samples for determination of these values were drawn from a random sample of men in the 30–39 year group immediately after the last blood specimen had been taken for hemoglobin determination in January 1961 After precipitation of the plasma with metaphosphoric acid, the ascorbic acid was estimated by titration with 2 6-di-chlorophenolindophenol sodium as an indicator

The serum-iron was assayed by Ramsay's method and the total iron-binding capacity of blood (TIBC) by the method given by Peters et al The blood samples for these determinations were taken in special tubes and examined at the Central Laboratory Ullevål Hospital.

Results

A Hemoglobin determinations

The mean hemoglobin values for the four test groups of men were fairly uniform for any particular month with no systematic inter-group differences (tables V and VI) The group means for the twelve-month period were also closely similar the highest and lowest means for the 30—39 year group differing by only 0.2 g, or 1 5 per cent, and those for the 50—59 year group by only 0.3 g or 2.0 per cent. If the two age groups are combined the following means are obtained

Ascorbic acid 15.2 g iron 15.2 g ascorbic acid + iron 15.3 g placebo 15.2 g

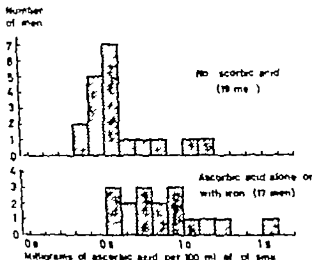


Fig. 1. Distribution of ascorbic acid content in plasma of men 30—39 years of age.

In the case of the school-children, too there were only minor differences between the four groups for any particular month (tables VII and VIII). The differences for the twelve-month period were also small. *It was usually the ascorbic acid + iron* groups that displayed the highest values. Since the hemoglobin values were no higher for the groups receiving ascorbic acid tablets than for the placebo groups, any difference may have been due to the extra supply of iron. While this cannot be ruled out, it is unlikely since the differences in favour of the group receiving iron tablets were quite small and did not increase over the twelve-month period.

The results thus indicate that for both the men and the school-children the hemoglobin levels were essentially unaffected by the supplementary ascorbic acid or iron or both, over a period of 12 months.

B. Ascorbic acid level in plasma

The ascorbic acid level in the plasma was determined for 36 men from the 30—39 year group, 17 of whom had received tablets containing ascorbic acid either alone or with iron, while the remaining 19 had received either placebo tablets or tablets containing iron but no ascorbic acid. From fig. 1 it can be seen that the ascorbic acid level in plasma *differed widely in the two groups* though being in general higher for the group of men that had received tablets containing 100 mg ascorbic acid. In view of the fact that a daily intake of 30 mg ascorbic acid — the recommended amount in Norway — would result in a plasma level of *at least 0.40* mg ascorbic acid per 100 ml (4), it would seem that some of the subjects did not receive the recommended amount through their ordinary diet.

C. Iron level in serum and the total iron-binding capacity of the blood (TIBC)

Results relating to serum-iron and TIBC were obtained from 39 men of the 30—39 year group, 18 of whom had received tablets containing 30 mg of

Table VII Mean Hb values (g/100 ml blood) for 10—13 year group of boys

Date	Placebo		Ascorbic acid		Iron		Ascorbic acid + iron	
	No.	Mean	No.	Mean	No.	Mean	No.	Mean
1960								
28—29/1	22	13.4	21	13.3	23	13.4	20	13.5
25—26/2	21	13.1	21	12.9	23	13.0	19	13.1
24—25/3	21	13.5	19	13.6	23	13.6	19	13.8
21—29/4	19	12.8	19	12.6	21	13.0	18	13.2
19—27/5	22	12.8	20	12.7	23	13.0	19	13.1
15—16/6	20	12.9	21	12.1	23	13.1	19	13.0
30—31/8	20	13.4	20	13.2	23	13.5	18	13.6
29—30/9	21	13.2	21	13.0	22	13.2	20	13.5
27—28/10	18	13.0	21	13.2	21	13.3	19	13.3
24—25/11	20	13.3	20	13.3	21	13.4	18	13.5
14—15/12	19	13.0	21	13.0	19	13.4	20	13.4
1961								
26—27/1	21	13.2	20	13.1	22	13.2	18	13.3
All observations	244	13.1	244	13.0	264	13.3	227	13.4

Table VIII Mean Hb values (g/100 ml blood) for 10—13 year group of girls

Date	Placebo		Ascorbic acid		Iron		Ascorbic acid + iron	
	No.	Mean	No.	Mean	No.	Mean	No.	Mean
1960								
28—29/1	19	13.3	18	13.3	19	13.3	17	13.4
25—26/2	17	12.9	18	12.7	19	12.8	15	13.1
24—25/3	19	13.2	18	13.2	17	13.2	16	13.4
21—29/4	17	12.8	17	12.8	17	13.0	16	13.2
19—27/5	18	12.6	17	12.6	17	12.6	17	13.3
15—16/6	17	12.9	18	12.9	19	12.9	17	13.1
30—31/8	18	13.2	18	13.2	19	13.3	16	13.3
29—30/9	17	13.2	18	13.1	18	13.3	16	13.3
27—28/10	17	13.0	16	12.9	15	13.1	17	13.6
24—25/11	16	13.1	17	13.2	17	13.3	15	13.6
14—15/12	16	13.0	16	12.9	15	13.0	15	13.1
1961								
26—27/1	16	12.9	18	13.1	14	13.4	16	13.2
All observations	207	13.0	209	13.0	206	13.1	193	13.3

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From the Institute of Hygiene, University of Oslo, Oslo, Norway

Studies on Hemoglobin Values in Norway

III. Seasonal Variations

by

HAAKON NATVIG, TOR BJERKEDAL and OYVIND JONASSEN

It has been shown by many earlier investigators that there are more or less marked seasonal variations in the hemoglobin content of the blood. The majority of the Scandinavian workers have found that the values are highest in the light season and lowest in the dark (3 4, 8, 9 10 12, 13 16, 22). Niels R. Finsen considered that this was in some way associated with the biological action of the solar radiation and that the seasonal variations would therefore be greater the higher the latitude. In other investigations the highest hemoglobin values have been recorded in the winter (6, 7 19 21) or in the utumn (5 14). This has been ascribed to nutritional factors, especially to higher content of iron and vitamin C in the diet t these seasons than at other times of the year

Many of the earlier studies on seasonal ariations in the hemoglobin content of the blood have been based on small numbers of cases, and the methods, by comparison with modern requirements, have left much to be desired. When account is taken of the variety of factors that may influence the hemoglobin values, the discrepant and occasionally contradictory results are not surprising. It was considered therefore that a closer study should be made of this problem, with a larger series of cases and better methods than have been used in many of the previous investigations.

Material and methods

The study was performed on healthy men and school-children at Kirkenes (latitude 70 N) and Oslo (60° N) 580 persons in all. The men t Kirkenes were employed at A/S Sydvaranger and those in Oslo t A/S Norsk Elektrisk Brown Boveri. All the men aged 30—39 and 50—59 years who at previous examinations by the works physician had been ecorded as healthy and who wer willing to participate were included in the study. Similarly all the healthy school-children in the third and fourth classes t the Kirkenes Elementary School, and t the Kampen Elementary School in Oslo who wer willing to participate were included in the study. The distribution of the subjects according to place of residence, age and sex is shown in tabl I.

Blood samples for the hemoglobin determinations were taken once a month at Kirke-

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Blood samples for the hemoglobin determinations were taken once a month at Kirke

Table I Number of adult men and school-children at Kirkenes and Oslo included in the study

Groups	Years	Kirkenes	Oslo
Men	30—39	176	37
Men	50—59	95	39
Boys	10—13	89	28
Girls	10—13	75	41
	Total	435	145

nes and Oslo over the period from January 1960 to February 1961 The samples were taken from the finger tip and the determinations were performed by the oxyhemoglobin method, with photoelectric reading on a Linson Junior apparatus the technique is described in an earlier article (20) At Kirkenes, all blood samples were taken and all readings performed by a specially trained nurse. In Oslo, blood sampling in respect of the men was performed by the works nurse and of the school-children by the school nurse. All the hemoglobin determinations on the men and school-children in Oslo were performed by one of the authors (H.N)

The number of subjects examined each month was not necessarily the same, owing to the fact that some had been absent on the day of the examination through for example disease, military service, civil defence exercises, holidays and business. A particularly large number were absent from the July examination. This variation may result in differences in the monthly means — for instance because those not examined may have been persons with low hemoglobin values. This possible cause of variation was avoided by calculating the monthly means on the basis only of persons who had been present at all the examinations.

Results

Men at Kirkenes (table II)

For the 1,975 hemoglobin determinations performed on the 30—39 year group at Kirkenes the annual mean was 15 3 g /100 ml of blood for the 50—59 year group 1 100 determinations gave a mean of 15 0 g The annual means for the 45 younger and 41 older men for whom a complete series of hemoglobin values was obtained are approximately the same as those for the whole groups.

The seasonal variations were, as can be seen from fig 1 roughly the same for the two age groups of men. Both curves show a fall from January 1960 to July after a rise from August to October the values remain at approximately the same level for the rest of the twelve month period For both groups the difference between the highest means, observed in January 1960 and the lowest observed in July 1960 is about 10 per cent.

School-children at Kirkenes (table III)

A total of 995 determinations on the boys gave an annual mean hemoglobin level of 13 2 g/100 ml, and 826 determinations on the girls a mean of 13 I The same respective annual means were recorded for the 45 boys and 35 girls on whom the full series of 12 hemoglobin determinations was performed. (Owing to the summer vacation no samples were taken in July)

The seasonal variations in the hemoglobin values for the boys and girls at Kirkenes was only 4—5 per cent (fig 1) The values vary irregularly especially during the first half of the year The highest values were recorded in March. The small variations have no counterpart in the curves for the adult groups at Kirkenes.

Men in Oslo (table IV)

The 465 hemoglobin determinations performed on the younger age group gave an annual mean level of 15.2 g/100 ml. The annual mean was the same for the 15 men on whom the full series of 13

Table II. Mean Hb values for men at Kirkenes for the 12-month period (g of Hb/100 ml blood) Figures in parenthesis are mean Hb values for 45 men aged 30—39 and for 41 men aged 50—59 years participating in each of the 13 determinations performed during the period of the study

Date	Men 30—39 years			Men 50—59 years		
	No.	Mean Hb value	S. D.	No.	Mean Hb value	S. D.
1960						
16—25/1	176	16.0 (16.1)	1.00	95	15.6 (15.6)	1.04
19—27/2	164	15.6 (15.8)	0.93	90	15.1 (15.2)	1.00
18—26/3	159	15.4 (15.6)	0.82	92	15.1 (15.2)	1.02
22—30/4	162	15.1 (15.2)	0.72	83	14.8 (14.8)	0.97
20—28/5	157	15.2 (15.4)	0.84	85	14.9 (14.9)	1.16
16—22/6	144	15.1 (15.1)	0.73	81	14.9 (14.9)	1.03
15—23/7	111	14.3 (14.3)	0.94	61	14.0 (13.8)	0.93
19—27/8	145	15.2 (15.0)	0.84	83	15.2 (15.0)	1.09
23—30/9	151	15.2 (15.2)	0.91	85	15.0 (15.1)	1.12
21—29/10	147	15.3 (15.5)	0.91	87	15.2 (15.1)	1.10
18—26/11	155	15.3 (15.4)	0.88	88	15.2 (15.1)	1.12
16—22/12	155	15.2 (15.3)	0.98	83	15.0 (14.9)	1.07
1961						
20—28/1	153	15.2 (15.6)	0.95	87	15.1 (14.9)	1.07
Total	1,973	15.3 (15.5)		1 100	15.0 (15.0)	

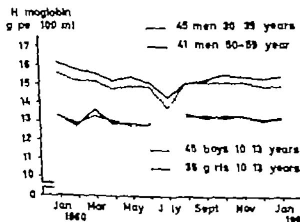


Fig. 1. Monthly mean Hb values for men and school-children at Kirkenes.

determinations could be performed. The 472 determinations on the older group gave a mean of 15.0 g/100 ml. For the 18 men on whom all 13 determinations were performed the annual mean was 15.1 g. As the curves in fig. 2 show the values for both groups decrease at the beginning of the year and remain low until May after which they rise again until December and January. The difference between the highest and lowest values is only 5 per cent. Neither of these seasonal

Table III Mean Hb values for boys and girls at Kirkenes for the 12-month period (g of Hb/100 ml of blood) Figures in parenthesis are mean Hb values for 45 boys and 35 girls participating in each of the 12 determinations performed during the period of the study

Date	Boys 10—13 years			Girls 10—13 years		
	No.	Mean Hb value	S. D.	No.	Mean Hb value	S. D.
1960						
28–29/1	89	13.4 (13.3)	1.18	75	13.3 (13.3)	1.12
25–26/2	87	13.0 (12.8)	0.80	71	12.9 (12.9)	0.70
24–25/3	85	13.6 (13.6)	0.90	72	13.2 (13.3)	0.63
21–29/4	80	12.9 (12.9)	0.72	68	12.9 (13.0)	0.68
19–27/5	87	12.9 (12.9)	0.95	71	12.8 (12.8)	0.65
15–16/6	84	12.9 (12.9)	0.73	73	12.9 (12.9)	0.64
30–31/8	81	13.4 (13.4)	0.60	71	13.2 (13.3)	0.52
29–30/9	84	13.2 (13.2)	0.76	69	13.2 (13.3)	0.76
27–28/10	79	13.3 (13.3)	0.86	65	13.2 (13.2)	0.78
24–25/11	79	13.4 (13.3)	0.88	65	13.3 (13.3)	0.66
14–15/12	79	13.2 (13.1)	0.80	62	13.0 (13.1)	0.71
1961						
26–27/1	81	13.2 (13.2)	0.80	64	13.1 (13.2)	0.76
Total	995	13.2 (13.2)		826	13.1 (13.1)	

Table IV Mean Hb values for men at Oslo for the 12-month period (g of Hb/100 ml of blood) Figures in parenthesis are mean Hb values for 15 men aged 30—39 and for 18 men aged 50—59 years participating in each of the 13 determinations performed during the period of the study

Date	Men 30–39 years			Men 50—59 years		
	No.	Mean Hb value	S. D	No.	Mean Hb value	S. D.
1960						
30/1	37	15.3 (15.6)	0.80	39	15.5 (15.7)	1.18
24/2	35	14.9 (14.7)	0.78	38	14.8 (14.6)	1.22
30/3	39	14.7 (14.5)	0.77	34	14.6 (14.6)	1.08
27/4	37	14.8 (14.8)	0.73	39	14.7 (14.7)	0.99
19/5	36	14.8 (14.8)	0.88	37	14.8 (14.4)	1.14
15/6	36	15.2 (15.0)	0.92	37	15.2 (15.3)	1.04
4/8	34	15.0 (15.1)	0.77	33	14.7 (15.0)	1.06
31/8	34	15.4 (15.5)	1.13	37	15.0 (15.3)	1.08
28/9	34	15.3 (15.4)	0.97	37	15.2 (15.4)	1.04
26/10	34	15.4 (15.3)	0.98	36	14.9 (15.0)	1.23
29/11	36	15.3 (15.2)	0.70	37	14.8 (15.0)	1.00
21/12	37	15.6 (15.7)	0.86	37	15.5 (15.7)	0.89
1961						
25/1	36	15.4 (15.4)	0.93	31	15.3 (15.4)	0.86
Total	465	15.2 (15.2)		472	15.0 (15.1)	

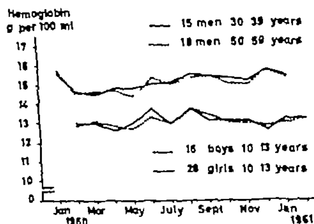


Fig. 2. Monthly mean Hb values in men and school-children at Oslo.

Table V. Mean Hb values for boys and girls at Oslo for the 12-month period (g of Hb/100 ml of blood). Figures in parenthesis are mean Hb values for 16 boys and 28 girls participating in each of the 13 determinations performed during the period of the study

Date	Boys 10—13 years			Girls 10—13 years		
	No.	Mean Hb value	S. D.	No.	Mean Hb value	S. D.
1960						
10/2	28	12.9 (13.0)	1.21	41	12.9 (12.9)	1.08
8/3	29	12.8 (13.0)	0.95	41	13.1 (13.1)	0.99
6/4	27	12.8 (12.6)	0.72	40	12.8 (12.8)	0.73
4/5	28	12.9 (13.6)	0.79	40	12.8 (12.7)	0.85
1/6	28	13.6 (13.7)	0.65	40	13.3 (13.3)	0.79
17/6	28	13.0 (13.0)	0.77	39	13.0 (13.0)	1.00
30/8	29	13.7 (13.7)	0.91	38	13.6 (13.7)	0.89
27/9	27	13.1 (13.1)	0.90	37	13.4 (13.4)	0.81
19/10	29	13.2 (13.1)	0.82	40	13.1 (13.0)	0.65
23/11	28	13.1 (13.1)	0.85	37	13.1 (13.0)	0.73
14/12	28	12.6 (12.6)	0.90	37	12.7 (12.7)	0.87
1961						
18/1	26	13.1 (13.2)	0.80	40	13.0 (13.0)	0.65
15/2	28	13.1 (13.2)	0.82	40	13.2 (13.2)	0.73
Total	363	13.1 (13.1)		510	13.1 (13.1)	

curves has the pronounced drop in the summer that was noted in the two corresponding Kirkenes groups.

School-children in Oslo (table V)

A total of 363 determinations performed on the boys and 510 on the girls both gave annual mean levels of 13.1 g. The same respective annual means were recorded for the 16 boys and 28 girls on whom all 13 determinations were performed. The curves for the seasonal variation in fig. 2 are irregular with maxima in June and August and minima

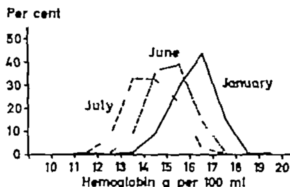


Fig 3 Distribution of Hb values for three months 50—59 year group of men at Kirkenes.

in April and December The greatest difference is, however only 7 per cent for the boys and 6 per cent for the girls. The curves have little in common with the corresponding ones for Kirkenes nor with those for the men at either Kirkenes or Oslo

Dispersion of the hemoglobin values in the various groups

The standard deviations (S D) relating to the observations for the individual months in the various groups of subjects are shown in tables II—V For men particularly the 50—59 year group the dispersion of hemoglobin values seems to be slightly greater than for boys and girls. Within each group of subjects, however there is little difference in the standard deviations of the observations for the individual months. This would suggest that differences between the monthly mean hemoglobin values are due to small changes in hemoglobin values to higher or lower levels for all or almost all subjects in the different groups, rather than to marked changes to particularly high or low values for a small number of subjects. This is illustrated in fig 3 showing the distributions of hemoglobin values for the younger men at Kirkenes for three selected months. The shift of the distribution for July towards lower values indicates clearly that the particularly low mean hemoglobin value recorded at Kirkenes that month was the result of a probably quite uniform drop in hemoglobin values in all the subjects. Similarly the high means for January may be ascribed to a general increase for the whole group

Discussion

If the hemoglobin level is influenced by the intensity of the solar radiation, a seasonal variation would be expected with higher values in the light and warm season than in the dark cold season moreover larger differences would be expected between summer and winter the further north the subjects lived. This was what Finsen supposed (9) The present study revealed no such seasonal variation. Among the men the highest values for both Kirkenes and Oslo related to the darkest and coldest winter months and the lowest values fell in July for Kirkenes and in March to May for Oslo

As regards the school-children, the highest values for Kirkenes were recorded in March, and for Oslo at the beginning of June and the close of August

Moreover there was no evidence of any other type of seasonal variation in hemoglobin level with consistently higher values in the autumn or winter as other authors have reported and which they have ascribed to dietary factors. As regards the diet, it has been demonstrated in an earlier study (20) that neither supplementary iron nor ascorbic acid had any influence on the hemoglobin values of men or school-children at Kirkenes, and

It is difficult to imagine that other dietary factors may account, wholly or in part, for the variations in the hemoglobin level.

While the investigation has indeed revealed a variation in the hemoglobin level over the year the differences between the months were small and displayed no consistent pattern of seasonal variation recognizable in all the groups examined.

It appears therefore that no seasonal variation in hemoglobin level — if this is defined as a biological variation that more or less consistently follows the seasons — was detected in either the Kirkenes or the Oslo groups. This suggests that the variations found also in this study are due to other factors that may be manifested at different times of the year

Among such factors are the voltage of the electrical supply the time of the day at which the blood samples were taken and its relationship to meal times, the posture during sample taking, and physical activity It is, however unlikely that these factors can have influenced the present results in view of the standardized blood sampling and apparatus used for the determinations.

A more likely cause of the differences observed is revealed by considering the conditions under which the particularly low hemoglobin values for men were obtained at Kirkenes in July This month determinations were performed during a period of days (15th to 23rd) with unusually warm weather Day temperatures of over 30° C in the shade were recorded and people found it so hot that those who could sat still in the shade and drank large quantities of cold beverages. Even at night it was so warm that many slept on their balconies. There is reason to suppose that this hot period was the most important cause of the low hemoglobin values recorded for the men at Kirkenes in July

It has been demonstrated that the blood volume increases in hot weather and decreases in cold this would result in lower hemoglobin values in the hot period and higher levels in the cold (2, 17) This is true, however only during the period of acclimatization. When heat or cold has lasted for 2—3 days, there will be a levelling out — that is, a return to normal values.

Bard (1) found that the blood volume was consistently higher in summer than in winter and that in the course of the first days after a change from cold to warm weather the blood volume could increase by 15—20 per cent and the hemoglobin value correspondingly decrease. This is in accordance with the strikingly low hemoglobin values found for persons examined at several business offices and blood donor centres in Oslo in the summer of 1955, when there was a heat wave in that part of Norway (11) Lange (15) considers the most important reason for the reports of seasonal variation in hemoglobin values lies primarily with the temperature factor

The increased intake of fluid in hot periods will increase the blood volume and thus cause a temporary drop in the hemoglobin level, the extent of which will depend on the quantity consumed and the rate it is drunk. In tests on himself Marx (18) found that the hemoglobin level increased by about 2 g after 4 days of thirsting and then fell 2 g after drinking 4.5 litres of water with 15.5 g of salt in the course of three days. One of Finsen's (9) subjects who had 123 per cent hemoglobin after a steam bath recorded 108 per cent hemoglobin one hour later after having drunk 600 g of water

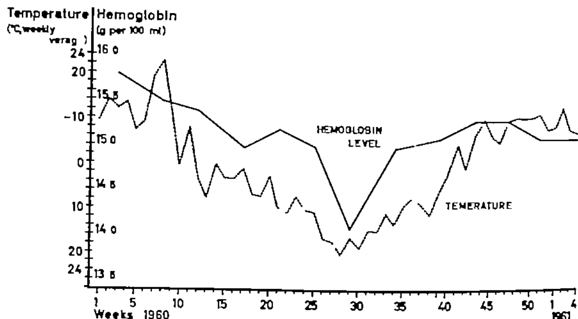


Fig 4 Relationship between mean Hb level and temperature 30—39 year group of men at Kirkenes

In view of these observations there can be little doubt that the low hemoglobin values for the men at Kirkenes in July were due to the unusually high outdoor temperature on the days the investigation was undertaken. There is evidence that the temperature may also be the reason for the fairly high hemoglobin values that were recorded during the cold season During acclimatization to cold the blood volume decreases and hence the hemoglobin level rises. Such an inverse relationship between the mean outdoor temperature and the hemoglobin level was found at Kirkenes In fig 4 which shows this relationship the lowest hemoglobin values coincide with the hot period and the highest levels coincide with the coldest period.

Since acclimatization both to warm and cold takes place over two or three days, the hemoglobin determinations must be performed on these days if any drop or increase in the values is to be detected. A coincidence of this nature seems to have been encountered during the sampling in July at Kirkenes, and explains the low values recorded. This may also explain why the same findings were not made for the school-children or the men in Oslo. An acclimatization to cold, with higher hemoglobin values, may of course also take place on a cold summer's day after a warm period while an acclimatization to heat with lower hemoglobin values might occur in the winter or spring and autumn for instance during mild weather with heating in the home, schools and working premises.

Summary

Seasonal variations in the hemoglobin level of the blood have been studied by determining the level every month over one year for 271 men and 164 school-children at Kirkenes (70° N) and 76 men and 69 school-children in Oslo (60° N)

Variations of 5 to 10 per cent in the mean monthly values were found. The high and low values, however appeared at different seasons for the different groups, and no typical seasonal variation

in, or characteristic annual pattern of, the hemoglobin values was found.

The study provides no evidence of greater seasonal variations in the hemoglobin level at the 70° latitude than at 60° nor that the hemoglobin level is higher in the light and warm season than in the darker and colder time of the year.

The study suggests that there is no true seasonal variation in the hemoglobin level, the small variations found probably being due chiefly to acclimatization to changes in temperature.

Acknowledgement

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Serum Enzymes after Cardiac Surgery

By

Kalevi Pyörälä, Ruben Gordin, Aarne Konttinen and Leo Telivuo

Application of enzyme determinations in the diagnosis of myocardial infarction focused attention on the changes of serum enzyme levels after cardiac surgery. In 1957 Crafoord et al. (7) and Schlief and Kamm (38) reported the first observations on the serum enzyme alterations after cardiovascular operations. Thereafter this subject has been dealt with in several studies (4, 6, 9, 17, 27, 33, 35, 39, 41, 46). Glutamic-oxalacetic transaminase (GOT) activity has been studied most extensively. In some studies alterations of lactate dehydrogenase (LD), glutamic-pyruvic transaminase (GPT) and some other enzymes have also been described. Some increase of GOT activity has been found to occur after all types of heart operations. Greatest elevations of GOT level, however, have been observed after open-heart operations performed under extracorporeal circulation. Increases of LD activity have been found to be smaller and no consistent changes have been observed in GPT activity.

Some investigators assume that great increase of GOT activity after surgical procedures involving direct heart trauma is due to a release of this enzyme from the heart muscle (9, 17, 30, 35). Snyder et al. (41) suggest hepatic changes produced by perfusion as the major source of the increased GOT values after operations performed under cardiopulmonary bypass, although they admit that in patients having operations by ventricular approach GOT may be released into circulation also from the heart muscle. Baer and Blount (4) and Werle et al. (46) are of the opinion that increased GOT levels and alterations of other serum enzymes cannot be ascribed to heart damage alone, but present the response of the whole organism to the stress of an extensive operation.

Recent advances in diagnostic enzymology have improved the accuracy of enzyme tests in the detection of myocardial lesions. Electrophoretic separation of LD isoenzymes, although too laborious

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Table I Group I Ligation of patent ductus arteriosus

Pat. no.	Age Sex	Serum enzyme levels on the 1st postoperative day (units/ml)				
		GOT	GPT	LD	HBD	CPK
1	32 ♂	50	11	300	236	39.0
2	16 ♀	42	12	400	415	15.4
3	18 ♀	48	13	325	161	22.3
4	30 ♀	48	13	350	212	12.4
5	21 ♂	68	14	400	183	35.1
6	22 ♀	60	12	375	132	18.0

for routine use, is useful in the differentiation of myocardial and hepatic damage (52) On the basis of observations of Rosalki and Wilkinson (36) on the relative activity of various LD fractions against pyruvate and α ketobutyrate, Elliott and Wilkinson (12) developed a new enzyme test in which α ketobutyrate is used as a substrate instead of pyruvate. The enzyme component converting α-ketobutyrate to α hydroxybutyrate has been called "α hydroxybutyrate dehydrogenase (HBD) The real nature of HBD is not definitely known so far it has not been possible to demonstrate HBD activity in the absence of LD activity (19 47 49) At any rate, the HBD test seems to be useful in confirming the diagnosis of myocardial infarction (11 12 13 20 24 25 31) Another enzyme test recently recommended for the diagnosis of myocardial infarction is the determination of serum creatine phosphokinase (CPK) CPK activity rises promptly after myocardial infarction and its measurement is considered to be the most sensitive among the enzyme tests used in the diagnosis of this disease (10 16 26 37)

The purpose of the present study was to get further information about the factors influencing the rise of serum enzyme activities after various heart operations. In addition to serum GOT and LD the new "cardiac enzymes HBD and CPK were studied. Serum GPT and alkaline phosphatase (AP) activities, as well as bromsulphalein (BSP) retention, were studied to obtain some information about the liver function during the post operative period

Material and methods

Three groups of patients undergoing cardiovascular operations were studied

Group I Six patients having ligation of patent ductus arteriosus

Group II Nine patients having mitral valvotomy

Group III Fifteen patients subjected to open heart operations under extracorporeal circulation for the correction of various congenital heart defects.

All the patients were adults the distribution of the series according to age and sex is shown in tables I—III.

N O—O — curare anaesthesia was employed in patients having ligation of patent ductus or mitral valvotomy Fluothane was used as an anaesthetic agent in patients operated on under extracorporeal circulation.

In cases of patent ductus arteriosus the chest was entered through a left posterolateral thoracotomy Left anterolateral thoracotomy was used in mitral valvotomy The surgeon explored the mitral valve with finger through an incision in the left atrial appendage and performed the valvotomy with the aid of the dilator designed by Tubbs. The dilator was introduced through a small stab wound at the apex of the left ventricle.

Table III shows the diagnosis and pertinent data concerning the operative procedure in the 15 patients operated on using extracorporeal circulation The Melrose-N.E.P disc oxygenator was used for cardiopulmonary bypass. In 11 cases operation was performed at normothermia or mild hypothermia and in four cases (patients 26, 27 28, and 30) at moderate hypothermia (+ 24 — + 30° C) In two cases of ventricular septal defect the temperature was reduced on perfusion until the heart stopped in ventricular fibrillation to facilitate the closure of the defect.

Table II. Group II. Mitral valvotomy

Pat. no.	Age Sex	Serum enzyme levels on the 1st postoperative day (units/ml)					Remarks
		GOT	GPT	LD	HBD	CPK	
7	45 ♂	75	15	263	293	40.2	Atrial fibrillation. Compensated right heart failure
8	35 ♀	63	11	600	217	19.7	Sinus rhythm. Compensated right heart failure
9	37 ♀	34	11	300	198	19.5	Sinus rhythm
10	38 ♀	80	15	608	388	26.6	Sinus rhythm
11	27 ♀	50	20	525	302	5.1	Atrial fibrillation
12	40 ♀	50	12	330	245	3.4	Sinus rhythm
13	43 ♀	36	13	375	393	4.7	Atrial fibrillation
14	51 ♀	75	17	330	430	18.8	Sinus rhythm
15	34 ♂	300	197	1,500	1,021	139.5	Atrial fibrillation. Multiple arterial emboli during operation. Died on the 4th postoperative day

Samples of blood for the determination of GOT GPT LD HBD and CPK were withdrawn before the operation and on the 1st, 2nd, 3rd, 5th, 7th, and 9th postoperative days. The serum was separated and stored at $-20°$ C, if the samples could not be processed immediately. The following methods were used in the determination of enzymatic activities: the method of Karmen et al. (22) for GOT, the method of Wróblewski and LaDue (51) for GPT, the method of Wróblewski and LaDue (50) for LD, the method of Elliott and Wilkinson (12) for HBD and the method of Tanzer and Gilvarg (43) for CPK. Reagents manufactured by AB Kabi, Sweden, were used in the determination of GOT activity and measurements of GPT LD and CPK activities were performed with the aid of laboratory kits manufactured by Biochemica Boehringer Germany. Enzymatic activities were expressed in spectrophotometric units. One unit is a change of 0.001 in optical density at 340 mμ per minute at a temperature of + 25 C in a cuvette with a light pathway of 10 mm. Serum alkaline-phosphatase (AP) activity was measured from samples withdrawn before the operation and on the 1st, 3rd, 9th and 11th postoperative days. Samples for the determination of this enzyme were processed immediately. The method described by Bessey et al. (5) was used and the results were expressed in Bessey-Lowry units (B-L units). The following upper limits of normal for the enzymes studied have been accepted in our laboratory: GOT 40 units/ml, GPT 35 units/ml, LD 500 units/ml, HBD 300 units/ml, CPK 4.0 units/ml, and AP 3.0 B-L units/ml. Bromsulphalein (BSP) retention test was performed before the operation and on the 1st, 3rd, and 7th postoperative days. Two mg of BSP per kg of body weight was administered intravenously and blood samples for the determination of plasma BSP level were taken 3 and 33 minutes after the injection. The accepted upper limit of normal is 6 per cent dye retention.

Results

The curves showing the mean values for the enzyme activities in the three groups of patients are presented in fig. 1

Table III Group III. Operations using cardiopulmonary bypass

Pat. no.	Age	Sex	Diagnosis	Type of incision	Duration of total bypass (min)	Minimum temperature (°C)
16	39	♀	Atrial septal defect	Bilateral transverse thoracotomy	21	33.2
17	43	♀	Atrial septal defect	Bilateral transverse thoracotomy	15	31.0
18	39	♀	Atrial septal defect	Midline sternotomy	43	34.8
19	32	♀	Atrial septal defect	Right anterolateral thoracotomy	40	32.0
20	33	♂	Atrial septal defect	Right anterolateral thoracotomy	38	33.8
21	27	♀	Atrial septal defect	Right anterolateral thoracotomy	36	31.9
22	20	♂	Pulmonary stenosis	Midline sternotomy	30	33.0
23	24	♂	Pulmonary stenosis	Midline sternotomy	15	33.8
24	23	♀	Pulmonary stenosis	Midline sternotomy	20	33.8
25	25	♀	Pulmonary stenosis	Midline sternotomy	17	33.3
26	22	♂	Ventricular septal defect (corrected transposition)	Bilateral transverse thoracotomy	70	28.0
27	17	♀	Ventricular septal defect	Midline sternotomy	30	25.4
28	33	♂	Ventricular septal defect	Midline sternotomy	76	24.0
29	49	♀	Pulmonary stenosis, Ventricular septal defect	Midline sternotomy	41	32.0
30	33	♂	Fallot tetralogy	Midline sternotomy	61	26.8

Serum enzyme levels on the 1st postoperative day (units/ml)					Remarks
GOT	GPT	LD	HBD	CPK	
50	19	713	407	27.4	—
156	29	500	320	46.2	—
158	18	725	434	23.1	Compensated right heart failure
63	17	400	502	61.6	—
88	14	513	232	15.4	—
125	23	1,250	323	29.0	—
175	24	358	491	61.6	Transarterial approach
63	18	158	416	12.3	Transarterial approach
113	20	313	217	20.1	Ventricular approach
94	19	467	329	10.3	Ventricular approach
450	21	1,225	1,463	34.0	Patch inserted in VSD. Short run of ventricular fibrillation at operation. Several periods of aortic clamping (total 15′10″)
225	13	330	614	33.2	Ventricular fibrillation induced by hypothermia
183	91	323	1,195	20.5	Ventricular fibrillation induced by hypothermia. Total heart block post-operatively. Treated with pacemaker. Normal a-v conduction returned on the 6th postoperative day
120	28	1,025	832	14.6	Compensated right heart failure. Two periods of aortic clamping (total 7′20″)
200	115	975	1,089	16.3	Total heart block postoperatively. Treated with pacemaker. Died of respiratory failure on the 4th post-operative week

Table III. Group III Operations using cardiopulmonary bypass

Pat. no.	Age	Sex	Diagnosis	Type of incision	Duration of total bypass (min)	Minimum temperature (°C)
16	39	♀	Atrial septal defect	Bilateral transverse thoracotomy	21	33.2
17	43	♀	Atrial septal defect	Bilateral transverse thoracotomy	15	31.0
18	39	♀	Atrial septal defect	Midline sternotomy	43	34.8
19	32	♀	Atrial septal defect	Right anterolateral thoracotomy	40	32.0
20	35	♂	Atrial septal defect	Right anterolateral thoracotomy	38	33.8
21	27	♀	Atrial septal defect	Right anterolateral thoracotomy	36	31.9
22	20	♂	Pulmonary stenosis	Midline sternotomy	30	33.0
23	24	♂	Pulmonary stenosis	Midline sternotomy	15	33.8
24	23	♀	Pulmonary stenosis	Midline sternotomy	20	33.8
25	25	♀	Pulmonary stenosis	Midline sternotomy	17	33.5
26	22	♂	Ventricular septal defect (corrected transposition)	Bilateral transverse thoracotomy	70	28.0
27	17	♀	Ventricular septal defect	Midline sternotomy	30	25.4
28	35	♂	Ventricular septal defect	Midline sternotomy	76	24.0
29	49	♀	Pulmonary stenosis, Ventricular septal defect	Midline sternotomy	41	32.0
30	33	♂	Fallot's tetralogy	Midline sternotomy	61	26.8

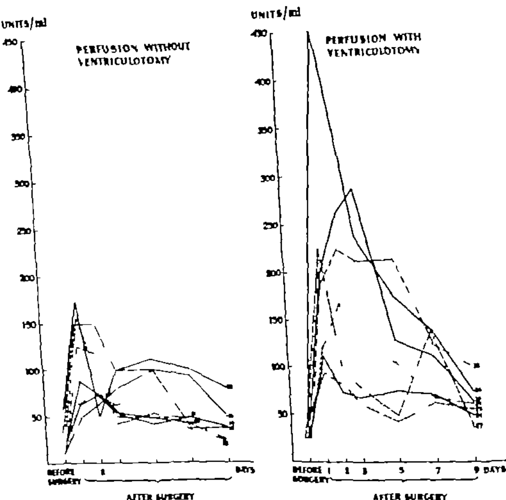


Fig. 2. Individual GOT patterns in patients operated on under extracorporeal circulation.

patients subjected to operations under cardiopulmonary bypass are given in figs. 2—4. In these figures the patients of group III have been divided into two subgroups 1) those having operations involving atriotomy or arteriotomy (patients 16—23) and 2) those having ventriculotomy (patients 24—30).

Group I. Ligation of patent ductus arteriosus

GOT activity rose slightly in all cases after the operation, the peak elevations ranged from 42 to 80 units. GPT activity showed slight elevation in two cases. LD activity remained within the normal range in all cases, whereas HBD activity showed slight elevation in four cases. CPK activity rose considerably in all cases, the peak elevations ranged from 12.4 to 39.0 units. Slight elevation of AP activity occurred in three cases on the 7th—11th postoperative days.

BSP retention rose slightly above the upper limit of normal in four of the six

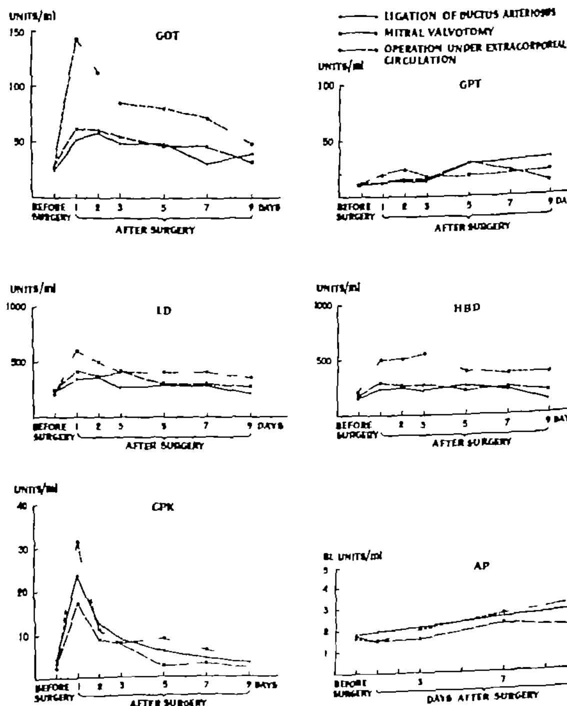


Fig 1 Mean values for the enzyme activities in the three groups of patients undergoing different kinds of cardiac surgery

Three patients with major complications, patients 15 28 and 30 (tables II and III) were excluded in calculating the mean values. To show the variation of the serum enzyme activities within each group, the individual values of GOT GPT LD HBD and CPK on the 1st postoperative day are given in tables I—III Complete data concerning the alterations of GOT LD and HBD

Group III Operations under cardiopulmonary bypass

A definite rise of GOT activity occurred in all patients. The mean GOT elevation in this group was distinctly higher than in patients subjected to ligation of patent ductus or mitral valvotomy (fig 1). Greatest GOT elevations were observed in patients who had operations involving ventriculotomy (fig 2). The highest GOT value, 430 units on the 1st postoperative day was observed in patient 26 with corrected transposition of the great vessels, who had closure of a large ventricular septal defect. Among the patients having operations by ventricular approach, the smallest GOT elevations occurred in patients 24 and 25 who had operations for relief of a valvular pulmonary stenosis. The peak GOT values in these cases were 113 and 94 units. Patients 22 and 23, who had a similar operation by transarterial approach, had GOT elevations to 175 and 75 units.

GPT activity rose during the immediate postoperative period in patients 28 and 30 in whom heart block developed during operation. The peak GPT values in these cases were 91 and 385 units. In two other patients a small temporary rise of GPT activity occurred during the second postoperative week.

Alterations of LD activity showed a great variability both in patients having operations without ventriculotomy and in patients operated on by ventricular approach, and there was no clear difference between these groups (fig 3). Increases of HBD activity were definitely greater in patients having ventriculotomy than in those operated on without it (fig 4).

CPK activity rose above the upper limit of normal in all cases the peak elevations ranged from 13.2 to 61.6

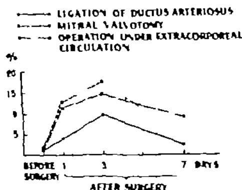


Fig. 5 Mean values for BSP retention in the three groups of patients undergoing various kinds of cardiac surgery

units. There was no definite difference between the patients operated on without ventriculotomy and those having ventriculotomy. The mean postoperative CPK values for the whole group were not definitely higher than those observed in patients having operations without cardiopulmonary bypass (fig. 1). AP activity was studied in 11 patients having operations under cardiopulmonary bypass. Slightly or moderately elevated values were observed in three patients on the 7th—11th postoperative days.

BSP retention was studied in 11 patients operated on using cardiopulmonary bypass. Abnormally increased values were observed after the operation in 10 cases the maximum values for BSP retention ranged from 4 to 59 per cent. The mean values are shown in fig 5.

When the duration of the total cardiopulmonary bypass in each case was plotted against the corresponding maximum values for the enzymes studied, correlations were found to be poor. When, however, the patients having ventriculotomy were considered alone, the maximum values of GOT and HBD were found to increase with the length of the

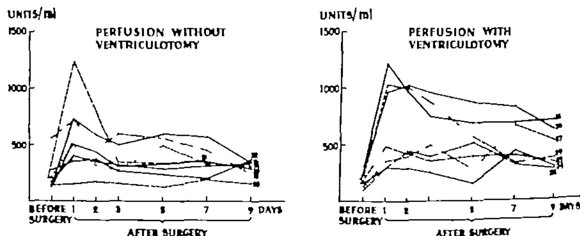


Fig 3 Individual LD patterns in patients operated on under extracorporeal circulation.

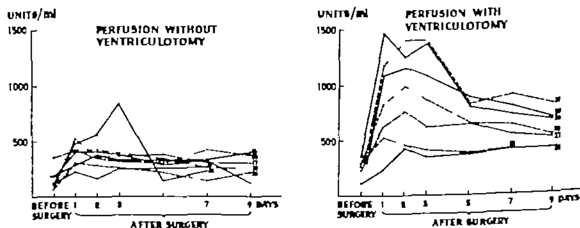


Fig 4 Individual HBD patterns in patients operated on under extracorporeal circulation.

cases the greatest value observed was 14 per cent dye retention. The mean values for BSP retention are shown in fig 5

Group II Mitral valvotomy

A slight increase of GOT activity occurred in all cases. In eight patients with uncomplicated postoperative course the peak elevations of GOT activity ranged from 43 to 112 units. A slight elevation of GPT activity occurred in two cases. Slight elevations of LD and HBD activity were observed in four cases. CPK activity showed elevated values in seven of the eight uncomplicated cases the maximum values ranged from 3 4 to 40 2 units. Slight increases of AP activity occurred in two cases. In patient 15 who had multiple emboli, the postoperative elevations of GOT GPT LD HBD and CPK were considerably greater than in uncomplicated cases (table II)

BSP retention was found to be abnormally increased after the operation in seven of the eight uncomplicated cases. The maximum rises ranged from 8 to 45 per cent. The mean values are shown in fig 5

by some degree of liver dysfunction (14 18, 23 40 42, 43) Crafoord et al. (7) have paid attention to the postoperative decrease of BSP clearance after operations performed using extracorporeal circulation. The decrease of the chromoexcretory capacity of the liver after various surgical procedures probably reflects mainly the effects of an impairment of liver circulation during and after surgery Experimental studies on portal haemodynamics during and after extracorporeal circulation (1 21 44) suggest that hepatic blood flow remains normal, if the perfusion rate approaches the basal cardiac output, whereas it is decreased at low flow rates. In the present series the flow rates employed during the cardiopulmonary bypass were slightly below the basal cardiac output in patients operated on under normothermic conditions and moderately decreased in patients having operations under hypothermic conditions. The results of BSP retention studies in the present series suggest that at least certain components of the liver function are impaired after various heart operations. However it seems improbable that liver damage could be the main source of the increased serum enzyme values. Hepatocellular damage causing GOT increases comparable to those observed after cardiopulmonary bypass operations could be expected to be accompanied also by some increase of the GPT activity In the present series no consistent GPT elevations occurred in patients with uncomplicated postoperative course. This is in agreement with the findings of Korberg and Senning (30) who also showed that serum ornithine carbamoyl transferase activity does not definitely increase after cardiopulmonary bypass operations. This enzyme is considered to be an even more sensitive indicator of liver injury than GOT and GPT (34)

Comparison of serum enzyme elevations after various types of heart operations may allow certain conclusions concerning the role of direct surgical heart trauma in the production of increased serum enzyme values. Ligation of a patent ductus involves no direct heart trauma and therefore the increases of GOT activity in patients having this operation must have some other explanation During mitral valvotomy performed by using a transventricular dilator an incision is made in the left atrial appendage, and, additionally a small stab wound is made in the left ventricle. However elevations of GOT activity after mitral valvotomy were not greater than those observed in patients having ligation of a patent ductus. Myocardial trauma produced during cardiopulmonary bypass operations performed by atrial or transarterial approach is not greater than that produced during mitral valvotomy Yet, several patients of this group had greater GOT elevations than patients having ligation of patent ductus or mitral valvotomy Evidently some other explanation than myocardial trauma must be sought also for the serum enzyme elevations occurring in patients having cardiopulmonary bypass operations without ventriculotomy Greater elevations of GOT activity were observed in patients having cardiopulmonary bypass operations involving right ventricular incision. Increased GOT values in this group of patients were accompanied by elevations of HBD activity If HBD activity in these circumstances provides a measure of the LD fractions originating from the heart muscle (48) the elevation of HBD level in patients having ventriculotomy sup-

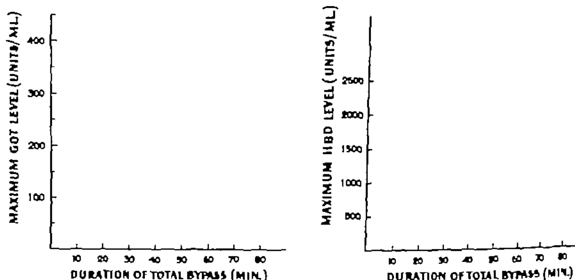


Fig 6 Correlation of the maximum GOT and HBD levels to the duration of the total cardiopulmonary bypass in patients having operations by ventricular approach.

total cardiopulmonary bypass (fig 6) The postoperative BSP retention values had no correlation to the duration of the bypass.

Discussion

An abundant literature is available on the alterations of GOT activity after various surgical procedures (e.g 2 3 8 15 27 28 29 32 45) GOT activity remains normal or is slightly elevated after abdominal surgery with the exception of gall bladder operations, that may produce considerably increased GOT values. Orthopaedic urological gynaecological and neurosurgical operations cause no alteration or a slight increase of GOT values. Thoracic surgery in general, produces somewhat greater GOT elevations than other kinds of surgery

Skeletal muscle trauma produced by thoracotomy incision evidently contributes little to the rise of the serum enzyme values, because in the present series the patients who had midline sternotomy in which no muscle was cut, had similar increases of the serum enzymes as the patients having muscle-cutting bilateral thoracotomies. A similar conclusion was made by Quinn et al. (33) and by Werle et al (46)

Norberg and Senning (30) studied serum enzyme alterations during the extracorporeal circulation itself and found an abrupt increase of aldolase, GOT and malate dehydrogenase, and a smaller increase of LD On the other hand Werle et al. (46) found only small increases of GOT activity during the bypass procedure. At any rate, the effect of the cardiopulmonary bypass on the cellular elements of the blood does not explain a rise of serum enzymes lasting for several days after the operation.

Snyder et al (41) suggested that reversible liver injury produced by extracorporeal circulation might be the main source of the increased GOT activity after cardiopulmonary bypass operations. Surgical procedures of much smaller extent than operations under cardiopulmonary bypass are known to be followed

Summary

Serum enzyme levels were studied in 30 adult patients subjected to cardiac surgery. Ligation of patent ductus arteriosus was performed in 6 patients, 9 patients underwent mitral valvotomy and 15 patients were subjected to open-heart operations under extracorporeal circulation for the correction of various congenital heart defects. The following serum enzymes were studied: glutamic-oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactate dehydrogenase (LD), α-hydroxybutyrate dehydrogenase (HBD), creatine phosphokinase (CPK) and alkaline phosphatase (AP).

A similar serum enzyme response was observed in patients having ligation of patent ductus and in patients having mitral valvotomy. A slight rise of GOT activity occurred in all patients. CPK activity showed a temporary rise to very high levels in most patients. Slight elevations of LD and HBD activity occurred in some patients. GPT and AP activities remained usually within the normal range.

Serum enzyme response in patients operated on under extracorporeal circulation resembled qualitatively that observed in patients undergoing cardiac surgery without cardiopulmonary bypass. Increases of GOT activity, however, were greater. Greatest elevations of GOT and HBD activity occurred in patients having operations involving ventricular incision.

Elevations of serum enzyme levels after cardiac surgery cannot be ascribed to any local tissue damage produced during surgery but evidently reflect mainly the effects of surgical stress on the whole organism. In patients having cardiopulmonary bypass operations involving ventriculotomy a release of enzymes from damaged heart muscle may contribute to the rise of serum GOT and HBD activity.

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ports the view that the heart muscle damage produced in these operations is great enough to be reflected in the serum enzyme values. The fact that the increase of HBD activity during the maximum rise of the serum enzyme values is relatively greater than the increase of LD activity provides additional support to this thesis (11)

The heart muscle trauma produced by surgical incision may not alone explain the great increases of GOT and HBD values observed in some patients having ventriculotomy In the two cases in which a short and simple procedure, pulmonary valvotomy was performed through the right ventricular incision increases of GOT and HBD levels were not greater than in patients having similar operations by transarterial approach. Moreover the maximum GOT and HBD levels in patients having ventriculotomy were found to increase with the length of the total cardiopulmonary bypass A similar observation concerning the postoperative GOT values was made by Fraser et al. (17) The occurrence of marked GOT and HBD elevations in patients subjected to long perfusion, however does not necessarily reflect the effect of the cardiopulmonary bypass itself on the heart muscle. There are several other factors that may increase the structural and metabolic damage in the heart muscle injured by surgical incision e.g manipulation and suture of the muscle tissue, disturbances of the heart rhythm, anoxia produced by intermittent aortic clamping etc. According to Werle et al. (46) the use of moderate hypothermia in connection with extracorporeal circulation does not modify the alterations of serum enzymes. Hypothermic cardiac arrest was employed in two cases of the present series. Although the serum enzyme alterations in these patients did not differ from those observed in other patients having comparable operations without arrest, no conclusions can be drawn about the effect of this procedure on the heart muscle. Potassium-induced cardiac arrest was found by Quinn et al. (33) to be an important factor in the production of marked GOT elevations. On the other hand according to Werle et al (46) and Fraser et al. (17) duration of cardiac arrest produced by anoxia has no definite effect on the postoperative GOT elevation

On the basis of evidence presented above it seems reasonable to assume that a release of enzymes from the heart muscle contributes to the rise of serum GOT and HBD levels after cardiopulmonary bypass operations involving ventricular incision It is, however not the only source for the increased serum GOT activity because definite GOT elevations occur also after operations involving no direct heart trauma or heart muscle damage too small to be reflected in the serum enzyme values. Evidently elevations of GOT activity after such operations as well as more inconsistent elevations of LD and HBD and great increases of CPK after all types of operations, are not due to any local tissue damage produced during surgery but reflect the effects of the surgical stress on the whole organism. The mechanism, by which the complex response of the organism produces elevated serum enzyme levels, is so far unknown Probably alterations in the distribution of blood flow during and after surgery lead to an impairment of circulation in certain readily vulnerable organs rich in enzymes and thus to disturbances in the cellular metabolism resulting in a release of intracellular enzymes into the blood stream.

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Types of Exudates in Diabetic Retinopathy

By

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The elements of the ophthalmoscopic picture of diabetic retinopathy are red dots ("microaneurysms") haemorrhages, dilated veins with irregular blurred contours (phlebopathy), macular pigment anomalies ("pigmentopathy of the macula lutea") (11) exudates and vascular proliferations with or without connective tissue formation.

Currently the exudates of diabetic retinopathy are described as small sharply demarcated yellow or white, waxy glistening patches, often coalescing into plaques. These exudates are usually termed "waxy exudates" or "hard exudates" because they have an appearance of hardness.

In patients with severe hypertensive retinopathy another kind of exudate occurs, namely the so-called "cotton wool exudate" or "soft exudate" (appearing soft). Such exudates can also be found in diabetic patients but it is generally thought that they occur only when the diabetes mellitus is complicated by arterial hypertension, and that they are the expressions of a hypertensive retinopathy often superimposed upon a diabetic retinopathy (1, 2, 7, 12, 15, 17).

However Hanum (9) mentions exudates "of a more grey colour and somewhat effaced, hazy limitations". Jahnert (10) states that "cotton wool exudates" occurred in 15 per cent of his cases of diabetic retinopathy and recently Wolter (18, 19, 20), Diezel and Willert (5) and Bloodworth (3) have discussed the histology of waxy as well as non waxy exudates in the retina of diabetics.

Some years ago we observed two patients whose eyegrounds were covered with exudates. These exudates looked like the "soft" or "cotton wool" exudates, but the patients' blood pressure was normal (14). Since then we have paid special attention to soft exudates in our diabetics and have gained the impression that they occur in many cases of diabetic retinopathy without hypertension. In some cases the colour of the exudate was brilliantly white, in others it was more greyish-white or greyish-red and quite indistinct.

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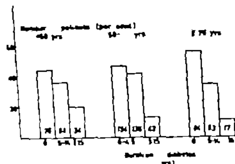


Fig. 2. Percentage of patients with various durations of diabetes in three age groups. The figures at the bottom are the absolute numbers, as in the following.

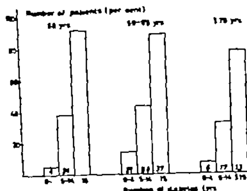


Fig. 3. Percentage of retinopathy in young, middle aged and old patients with varying duration of diabetes.

of years from the time the diagnosis was first made, appears from fig. 2. The incidence of 0—4 years diabetes and 5—14 years diabetes is about the same in young, middle-aged and old patients, but there are fewer patients with more than 15 years of diabetes (long-term diabetics) among the old. This pattern was the same for *men* and *women* and nearly the same in the city and county series.

The overall percentage of long-term diabetics was 14.

Retinopathy

The ophthalmoscopic diagnosis of diabetic retinopathy was based on the commonly accepted criteria, i.e. the presence of red dots ("microaneurysms") and/or phlebopathy and or hard exudates and/or vascular proliferations.

Several kinds of these elements were present in most cases, but not in all. Retinopathy was diagnosed solely on the basis of red dots ("microaneurysms") in 22 per cent of the retinopathies. In 8 per cent it was based on phlebopathy alone. Only one patient had hard exudates without any other elements and isolated proliferations did not occur in this series.

It seems worth mentioning that the incidence of the individual elements of diabetic retinopathy noted by each of the three authors was about the same.

Retinal haemorrhages were not included in the diagnostic criteria, because they have *no characteristic morphological features* in diabetes. They occurred in 66 per cent of the cases of diabetic retinopathy according to the definition as against 3 per cent in patients without retinopathy and were seen with the same frequency in patients with hard, soft white or soft grey exudates.

Vascular proliferations — with or without connective tissue formation — occurred in 10 per cent of the cases of retinopathy.

The overall incidence of diabetic retinopathy without regard to age or duration of diabetes, was 32 per cent.

Fig. 3 shows the correlation between diabetic retinopathy, age and duration of diabetes. As was to be expected, the incidence rises with increasing duration of diabetes mellitus. This is true in all

The following is a report of a systematic study of the incidence of various kinds of exudates in diabetic retinopathy and of the relationship between types of exudate and other aspects of the diabetic angiopathy

Material and methods

The study comprises 717 diabetic patients. In 499 patients living in the city of Aarhus the ophthalmoscopic examination was performed by two of us (V E. and K. L.) 218 patients from the county of Aarhus were examined by P H M

The Zeiss hand ophthalmoscope (cat. no. 300 701 Transformer cat. no. 309,227) with green filter was used throughout. In this way powerful illumination and good colour contrasts are obtained, permitting considerably more detailed observations than with the current battery hand ophthalmoscopes.

The ophthalmoscopic examination usually lasted from 10—20 minutes as it was necessary to find and to note all abnormalities, even very small ones. The results were recorded on special cards.

Retinal photographs were taken in cases where exudates were found by ophthalmoscopy

The *exudates* observed were classified according to the following definitions

1 *Hard exudates* yellow or white waxy sharply demarcated glistening patches about as large as the diameter of a retinal artery one or several in clusters or plaques.

2 *Soft white exudates* white cotton wool like patches with blurred and hazy edges, about half the size of the papilla or smaller

3 *Soft grey exudates* like cotton wool exudates, but greyish in colour and faintly seen. Often nearly invisible without green filter

It was easy to distinguish between hard and soft exudates, but in some cases it was difficult to decide if a larger exudate with hazy contours should be classified as a soft grey exudate.

Fig 1 shows characteristic examples of these three types of exudates. As was to be expected it was not easy to obtain good photographs of the soft grey exudates.

In a few cases we have observed exudates which did not look like either of the three types described here, such as larger irregular areas that seemed to be covered by a fine grey veil. These changes will not be discussed in the present paper

The *blood pressure* was measured on the right arm after the patient had been reclining on a bed for 5 minutes. Readings were made to the nearest value divisible by 10. The diastolic pressure was read when the character of the sound changed

Urine protein was determined by Albustix. If positive, a quantitative determination was performed.

Serum cholesterol phospholipids and *total lipids* were determined by the method of Schoenheimer and Sperry (16)

Results

General analysis of the series

The 499 patients from the city of Aarhus were selected arbitrarily among the patients visiting the Diabetic Clinic between April 1960 and January 1962. A statistical analysis showed that this series was representative as to sex and age of the total diabetic population of the city (about 700)

In 40 of these 499 patients opacities of the ocular media prevented ophthalmoscopic examination on one or both eyes. The distribution of the remaining 459 patients in the various sex and age groups was the same as in the total group

The 218 cases from the county of Aarhus were the patients admitted to the medical and surgical departments of the Aarhus County Hospital between February 1960 and February 1961 There were more old patients in this group than in the group of city patients, but the difference was only small

In 15 of these 218 patients ophthalmoscopy was not possible.

The duration of diabetes mellitus in the various age groups, i e the number

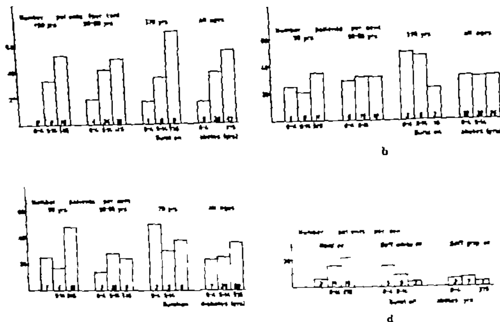


Fig. 4. Percentage of hard ( ) soft white (b) and soft grey ( ) exudates in young middle aged and old patients with retinopathy The last block of columns shows the results for all ages put together (d) Isolated exudates, all ages.

Red dots (microaneurysms") and exudates

It appears from fig. 5 that there is a correlation between the incidence of exudates and the degree of diabetic retinopathy as it is expressed by the number of red dots in the fundus the incidence of exudates is higher the higher the number of red dots. This correlation is found for hard as well as for soft white and soft grey exudates.

Blood pressure and exudates

Fig. 6 a and b shows the percentage distribution of three types of exudates in patients with different levels of blood pressure.

Hard exudates were found more often in patients whose diastolic pressure was 100—110 mm than in patients with a lower blood pressure ($p < 0.01$) The same tendency appears for the systolic pressure, but the differences are not statistically significant. The number of patients with high blood pressure (diastolic =/> 120 or systolic =/> 210 mm) is so small that no conclusions can be drawn.

There is no statistically significant difference between the incidence of soft white or soft grey exudates in the various blood pressure groups.

The calculations of these distributions were performed — just like those of the preceding section — on the basis of each type of exudate, whether it occurred as an isolated phenomenon or together with one or both of the other types. An analysis of isolated types confirms the result that waxy exudates are seen more often

Table I Three types of retinal exudates isolated and in various combinations

	No. of cases	%
Hard exudates	36	29
Soft white exudates	17	13
Soft grey exudates	12	10
Hard + soft white	13	10
Hard + soft grey	10	8
Soft white + soft grey	11	9
Hard + soft white + soft grey	27	21
Total	126	100

age groups and in particular it should be noted that the incidence of retinopathy in patients with long term diabetes (≥ 15 years duration) is nearly the same in the old as in the young patients.

These distributions were the same in the city and the county series except for a somewhat higher incidence of retinopathy in the middle aged patients with diabetes of 5—15 years duration *in* the county-series

It appears that in the analysis of age, sex, duration of diabetes and incidence of retinopathy there were only small and insignificant differences between the two series, that of the city and that of the county populations. Moreover the study of the types of exudates and their correlations gave nearly identical results in the two series In the following therefore the results of the analysis of the 662 patients in the combined series will be presented.

Types of exudates

Exudates of the types under discussion were present in 142 of the 662 patients. They occurred in only 16 cases who did not have retinopathy according to the definition i e. in 3 5 per cent of the patients without diabetic retinopathy There were five cases of soft white exudates and eleven cases of soft grey exudates. Nearly all of these cases were old patients, some with a recently discovered diabetes and some who had had diabetes for many years.

One hundred and twenty-six of the 211 patients with diabetic retinopathy had exudates, i e. 60 per cent. The occurrence of isolated or combined types of exudates appear from table I It is seen that in 32 per cent of the retinopathies with exudates these were *not* of the hard or "waxy" variety

Fig 4 a shows the percentage distribution of the 86 cases with *hard exudates* in the same age and duration-groups as in the above analyses. It appears that there is a rise in the incidence of these exudates with increasing duration of diabetes mellitus. This tendency is the same in all groups but it should be noted that the absolute figures are quite small in the oldest age-group

Fig 4 b gives the corresponding results for the soft *white exudates* Here the incidence in the various groups is about the same, and the last set of columns showing the overall results without regard to age, shows no difference at all

The same applies to the distribution of the soft grey exudates (fig 4 c)

The distributions shown in fig 4 a—c are calculated on the basis of patients presenting hard soft white or soft grey exudates whether they had more than one type of exudates or not. Fig 4 d shows the distribution when all patients are excluded who had more than one type of exudate The rising incidence of hard exudates with increasing duration of diabetes is found again, but isolated soft white or soft grey exudates seemed to be less common the longer the diabetes has lasted.

patients with retinopathy than in patients without, and especially so in patients with hard exudates, but these differences are not statistically significant.

There were no significant differences between the concentrations of the various fractions in patients with or without soft white or soft grey exudates.

The values obtained in the smaller county-series showed the same tendencies as in the city-series, but on statistical analysis these differences were not significant.

Repeat examination

In a few patients with very pronounced exudates the examinations were repeated 6—18 months later and the observations were carefully compared with those of the first examination.

In one patient one small area of hard exudate noted at the first examination could not be found at the repeat-examination. All other areas of hard exudate were unchanged or had increased in size.

On the other hand many soft white and soft grey exudates had disappeared and new ones were observed that had not been present at the first examination. In some cases an exudate which had been classified as soft white at the first examination now had the typical appearance of a soft grey exudate, and *vice versa*.

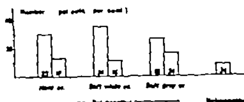


Fig 7 Percentage incidence of proteinuria in patients with and without exudates

Discussion

The term "exudate" has been used since the earliest days in the history of ophthalmoscopy The word implies a "sweating out" or deposition of some substance in or on the retina but as a designation of an ophthalmoscopical phenomenon it hardly denotes more than a change of colour In the examinations and analyses presented in this paper "exudate" only means a localized change of the normal red colour of the retina in

Table II Serum lipid fractions in patients with or without retinopathy and the three types of exudates Average figures and S E. of mean. Statistically significant differences. 1 2 cholesterol, $p < 0.05$ 2 3 cholesterol p 0.01

		No.	Cholesterol	Phospholipids	Total lipids
1	+ Retinopathy	136	273 ± 5.0	248 ± 4.1	992 ± 18.2
2	— Retinopathy	305	261 ± 3.3	241 ± 3.3	960 ± 13.8
3	Hard exudates	55	283 ± 7.6	253 ± 6.9	1,009 ± 30.3
4	— Hard exudates	81	270 ± 6.6	245 ± 2.5	979 ± 22.7
5	+ Soft white exudates	43	272 ± 8.5	247 ± 7.0	982 ± 30.5
6	— Soft white exudates	93	277 ± 6.2	248 ± 5.2	996 ± 22.7
7	+ Soft grey exudates	34	267 ± 11.0	241 ± 8.4	962 ± 37.0
8	— Soft grey exudates	102	278 ± 5.5	250 ± 4.8	1,001 ± 20.9

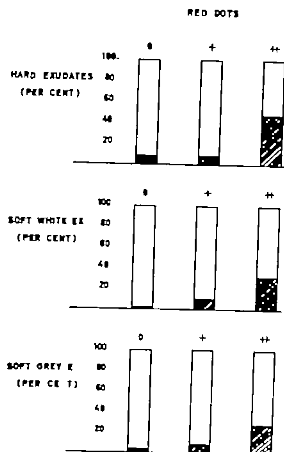


Fig 5 Occurrence f the three types of exudates in patients without red dots (microaneurysms") with 1—2 (+) or w th more than 2 dots (++)

in patients with a high than with a lower blood pressure. They occurred in 4 per cent of patients with a diastolic pressure </ = 90 mm, but in 9 per cent of the patients having a diastolic pressure of 100—110 mm. This difference is statistically significant ($p < 0.025$) The correlation between isolated hard exudates and systolic pressure is also statistically significant.

On the other hand there was no correlation between isolated soft white or soft grey exudates and the blood pressure.

Proteinuria and exudates

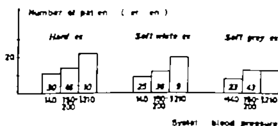
As was to be expected proteinuria was more frequent in patients with retinopathy than in patients without. The incidence was greater in patients with than in patients without exudates. This was true of all three kinds of exudate (fig 7)

Serum lipid fractions and exudates

Table II gives the results of the determinations of serum cholesterol phospholipids and total lipids in the city group

Serum cholesterol is significantly higher in patients with than in patients without diabetic retinopathy This difference is more pronounced when patients without retinopathy are compared to patients with hard exudates ($p < 0.01$) On the other hand if cases with hard exudates are excluded there is no significant difference between patients with and patients without diabetic retinopathy

The average values of phospholipid and total lipid of the serum are higher in



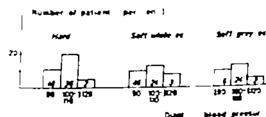


Fig 6 a and b. Percentage of cases of retinopathy w th varying blood pressures ha ing hard, soft white or soft grey exudates.

It has been shown earlier that the serum cholesterol tends to be higher in patients with than in patients without diabetic retinopathy (15) This was confirmed in the present study and it appeared that this correlation was due to a connection between hard exudates and the serum cholesterol level

The average values of serum phospholipids and total lipids showed the same pattern, but here the differences were not statistically significant.

It therefore seems that the concentration of cholesterol in the blood of diabetic patients — perhaps also of other lipid fractions or of the physical state of these lipids in the blood — may play a role in the development of the hard retinal exudates. That this might be so is also revealed by the fact that such exudates can be eliminated from the retina by treatments which cause a fall of serum cholesterol. Disappearance of hard exudates have been observed during treatment with a low fat diet (6) a diet rich in unsaturated fatty acids (13) and by administration of para-aminosalicylic acid (8)

Soft white or grey exudates were not found to be correlated with th duration of diabetes, the blood pressure or the serum cholesterol. In a few cases examined repeatedly these exudates were found to vary a good deal from time to time. It may be thought, therefore, that they ar dependent upon the momentary metabolic of the retina but the fact that they are more common in the retina if many red dots" are present than if there are only few demonstrates that soft exudates are also related to the vascular disease as such. The same appears from the orrelation between the incidence of proteinuria and the incidence of exudates. This correlation obtains for hard a well as for soft white or grey ...

It is possible that small thrombi occur in the development of vascular disease in the retina, and that these are the cause of the soft exudates. In the initial stages the microinfarcts formed may be accompanied by oedema, making them appear as soft exudates on ophthalmoscopy

In all the analyses of the present results it was found that hard exudates differed from soft white or soft grey exudates but no difference has appeared between the two forms of soft exudates. This indicates strongly that the white and the grey soft exudates are various forms of one and the same retinal anomaly It seems reasonable, therefore, to distinguish only two types of exudates in diabetic retinopathy viz. *hard exudates* and *soft exudates* (white or grey)

The soft exudates are particularly interesting, as they may depend on factors other than the development of the long-term diabetic angiopathy A systematic follow-up study of patients with soft exudates would supplement the snap shot results obtained in the present study It would seem to be of some interest to study if these exudates can be made to vary in accordance with variations in the blood sugar level.

However a deeper understanding of the problems of retinal exudates in diabetic retinopathy requires histological and histochemical studies of various kinds of exudates, preferentially upon autopsy material from normotensive patients ophthalmoscoped shortly before death.

Summary

Diabetic retinopathy occurred in 211 of 662 diabetics examined ophthalmo-

the direction of white, yellow or grey as observed with the ophthalmoscopic set up chosen for this study

Information about the histology and histochemistry of retinal exudates in diabetic patients is rather scarce. Hard exudates are situated in the outer reticular layer of the retina. From their staining reactions they are thought to be homogeneous depositions of lipoproteins and mucoproteins (3 5 18)

Histologically the cotton wool exudates of hypertensive retinopathy are made up of clusters of pear-shaped bodies ("cytoid bodies) in the nerve fiber layer The nature of these cytoid bodies have been discussed for many years Wolter (19 20) using silver stains have found them to be swollen and curled up interrupted axons. Christensen (4) believes that they are precipitates of ground substance and fibrin derived from the blood stream

The nature of the white and grey exudates described in the present report are not known. Diezel and Willert (5) Wolter (19) and Bloodworth (3) have described "cytoid bodies in the retina of some diabetic patients, but the blood pressures of these patients are not stated.

In the present study we endeavoured to distinguish between three types of exudate according to the ophthalmoscopic picture 1 *hard exudates* i.e the small white or yellow waxy glistening sharply demarcated exudates usually described in the textbooks. 2 *soft white exudates* that differ from the hard exudate by their size and by their hazy contours. Usually only a few of these exudates occur in one fundus and they are considerably larger than the hard exudates. 3 *soft grey exudates* that look like the white ones but are more greyish or greyish red in colour

The ophthalmoscopic reality of this classification is evident from the fact that the incidence of the three types was the same in the groups of patients examined by each of the three authors.

Exudates very rarely occur in diabetics without the usual signs of diabetic retinopathy In patients with retinopathy they are found in 60 per cent of the cases. This incidence is one and a half times higher than that in an earlier study (15) The reason for this difference might be that more attention was paid in the present study to other exudates than the hard ones but probably also that a better ophthalmoscope was used. Hard exudates were predominant in the present series but it was surprising to find that not less than one third of the cases with exudate did *not* present this classical type of diabetic exudate.

The incidence of hard exudates increases with increasing duration of diabetes mellitus This confirms the general experiences that these exudates seldom or never disappear once they have been found. On the other hand the incidence of soft white and soft grey exudates was not dependent on the duration of diabetes This suggests that these exudates are transitory features in the retina.

Hard exudates occurred in patients with a high as well as in patients with a lower blood pressure, but there was a positive statistical correlation between the incidence of hard exudate and the height of the blood pressure. No such correlation was found in the analysis of the soft white or soft grey exudates. It is clear therefore, that these soft exudates that look like the wellknown cotton wool exudates of non-diabetic hypertensive patients are not caused by elevation of the blood pressure.



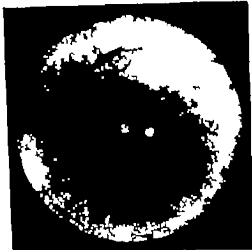




Fig. 1 a. A group of hard exudates between the papilla and the macula. One soft white exudate. b A soft white exudate. c. A soft grey exudate, faintly visible in the centre of the figure.

patients with retinopathy showed retinal exudates.

Ophthalmoscopically three types of exudates have been distinguished in the present study viz hard exudates ("waxy exudates) soft white exudates ("cotton wool exudates) and soft grey exudates. However the results obtained suggest that there is no reason to distinguish between soft white and soft grey exudates

Thirty two per cent of the exudate cases did not show the classical hard or waxy exudates currently described in diabetic retinopathy

The incidence of hard exudates is correlated with the duration of diabetes the blood pressure and the serum cholesterol level The soft exudates showed no such correlations

Hard exudates seem to be a feature in the slow and insidious development of the long term diabetic angiopathy

Soft exudates must also be expressions of this development since they seldom occur in diabetics without retinopathy They may be the results of microthrombi or may reflect changes in the momentary metabolic state of the retina.

Acknowledgment

W wish to express our ppreciation to Professor P Bechgaard M. D. and chief surgeon P Holm-Nielsen, M. D., heads of the medical and of the surgical wards of the Aarhus County Hospital, for letting us examine their patients.

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Acta Medica Scandinavica. Vol. 174, fasc. 4, 1963

From the Medical Out-Patient Clinic, University Hospital, Copenhagen, and the Protein Laboratory, Institute of General Pathology, Copenhagen, Denmark

Urinary Protein in Glomerulonephritis and Pyelonephritis

Electrophoretic Analyses

By

[illegible]

It has come to be universally recognized that normal humans excrete protein even while resting in bed. Accounts have been given in previous papers of quantitative as well as qualitative investigations into the protein in normal urine. The amount of protein excreted per hour has been found to be under 2.5 mg. The qualitative protein analyses have been carried out by means of paper electrophoresis of urine after concentration by positive pressure dialysis (7, 8, 9). Normal urine contains approximately equal amounts of albumin and globulin. The globulin fraction is not differentiated into α-, β- and γ-globulin. Fig. 1 presents a few patterns for normal urine.

While only few investigations are available into the protein of normal urine, many have been published concerning the protein excreted in glomerulonephritis and in other diseases with a high protein concentration in the urine. The analytical methods have been those of free electrophoresis, paper electrophoresis, and in a few instances immunoelectrophoresis.

Below an account will be given of electrophoretic analyses of the urinary protein in glomerulonephritis, in pyelonephritis, and in a few other renal diseases with proteinuria. No regard was paid to whether the urine contained large or immeasurably small amounts of protein as judged by Heller's reaction. The urinary protein was in all the cases concentrated as described previously (7, 9).

In cases of pronounced proteinuria the amount of urine used was 10 ml, in mild cases (about 10 mg/100 ml of urine) 25—50 ml, and in Heller-negative urine specimens 100 ml.

Results

Glomerulonephritis

Analyses of urine specimens from 21 patients suffering from glomerulonephritis gave electrophoretic patterns as shown in figs. 2 and 3. The curve for the albumin fraction was high and sharply peaked in all the cases. The globulin fraction gave a low curve, as a rule with a marked apex within the β-range. The

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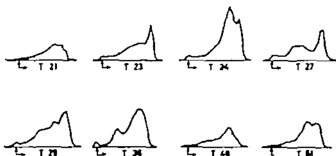


Fig 1 Electrophoretic patterns for normal urine. The areas of the curves indicate the amount of protein in the urine concentrate.

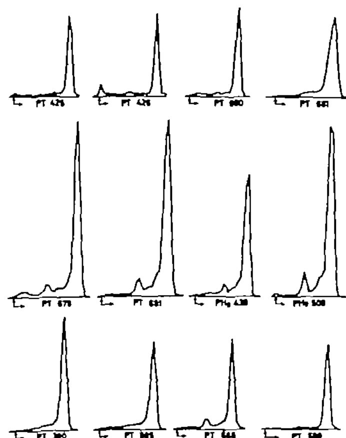


Fig 2. Electrophoretic patterns in glomerulonephritis (the areas of the curves indicate the amount of protein in the urine concentrate) PT 425—426 and PT 660—661 are patterns representing diurnal and nocturnal urine specimens. PHo 509 is from a patient with genuine nephrosis. The protein excretion was within the normal range in PT 661 and PT 390. I PT 426 and PT 585 the excretion was 200—300 mg/ 1,000 ml The remainder excreted over 1,000 mg/1 000 ml.

curve for the γ-fraction was very low in nearly all the cases while that for the α fraction varied somewhat in size As stated by other writers (1 2 3 4 5 6) a large α-fraction is found particularly at the nephrotic stage. In the present series the appearances of the patterns seemed to bear no relation to the stage of the disease or the degree of proteinuria. In one patient, however during the aggravation of the disease the last week before death there was found an increasing division of the globulin fraction which at the same time grew in size, finally to resemble the serum-electrophoretic pattern of the patient (fig 3) A similar picture was observed later in another patient with clinical manifestations of the

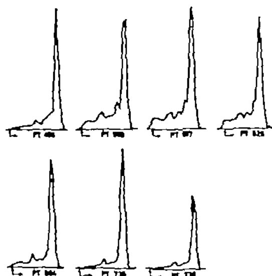
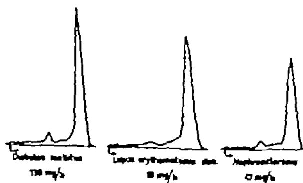


Fig. 3. Electrophoretic patterns in glomerulonephritis (the areas of the curves indicate the amount of protein in the urine concentrate). PT 496—509—517—526 are from the same patient the last few weeks before death. PT 526 represents the analysis the day before death, when the globulin fraction had increased and split like serum protein (cf. text). PT 735—736 represent diurnal and nocturnal urine specimens.

The protein excretions ranged between 1,300 and 5,000 mg/1,000 ml.



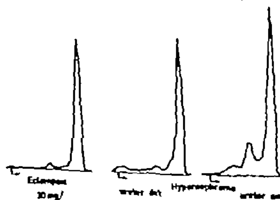


Fig. 4. Electrophoretic patterns in different forms of proteinuria. The protein excretion per hour is stated in the individual cases. The patient with hypernephroma excreted less protein from the right kidney (the site of the hypernephroma) (240 mg/1,000 ml against 1 450 mg from the left kidney.

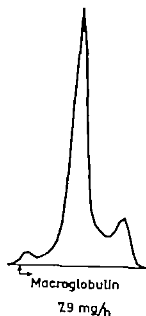


Fig 5 Electrophoretic pattern in a case of macroglobulinemia Waldenström. The protein excretion was 7.9 mg per hour The abnormal fraction migrated at the same rate as the abnormal fraction in serum

same kind. In no other cases at the terminal stage were such changes noticed in the electrophoretic patterns.

In most other forms of proteinuria the electrophoretic patterns resemble those shown in fig 2 and fig 3 with a high, pointed curve for the albumin fraction followed by a low curve for the globulin fraction, as a rule with a rather marked β-fraction Fig 4 gives a few instances of analyses of urine specimens from patients with diabetes mellitus, lupus erythematosus nephrosclerosis, and eclampsia.

All the analyses were carried out on urine passed spontaneously by the patient. In a single case, a patient with right hypernephroma without grossly visible hematuria, ureteral urine specimens from both the right and the left side were analysed The result is shown in fig 4

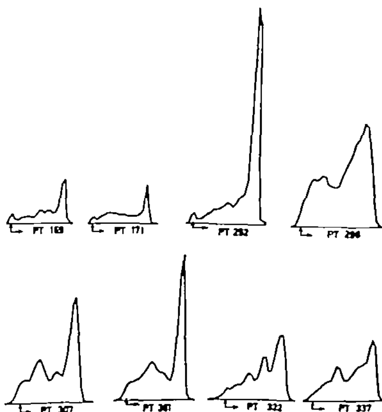


Fig 6. Electrophoretic patterns in pyelonephritis (the areas of the curves indicate the amount of protein in the urine concentrate) PT 307—361 are from the same patient, whose urine was analysed with an interval of one month. PT 322—337 are from another patient checked after one month. PT 232 excreted 1 117 mg/1,000 ml or 135 mg/hour PT 169 excreted 23 mg/hour and PT 171 40 mg/hour In the remaining cases the protein excretion was under 15 mg/hour i. e between 100 and 200 mg/1 000 ml.

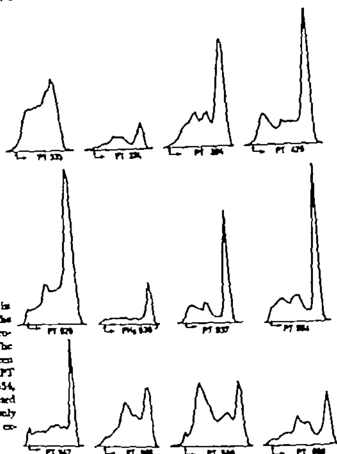


Fig 7 Electrophoretic patterns in pyelonephritis (the areas of the curves indicate the amount of protein in the urine concentrate). The protein excretions ranged between 400 and 1,700 mg/1,000 ml in PT 475 — P Ho 530 — PT 537 554, and 547 The remainder excreted about 100 mg/1,000 ml, i. e. only just above the limit of normal excretion.

Even if the protein excretion presents no marked pathological rise in a patient, a renal disease can sometimes be disclosed by electrophoretic analysis of urine for protein In a patient with latent glomerulonephritis the graph will then show a high albumin peak and a small globulin fraction, a pattern differing markedly from that for normal urine. In cases where abnormal proteins are present in serum, these will also occasionally be present in the urine even at a very low protein excretion. Fig 5 illustrates a urine specimen from a patient with macroglobulinemia Waldenström (protein excretion 7.9 mg/hour) The abnormal protein fraction was here plainly visible in the urine. In this case it migrated at the same rate as the abnormal serum protein.

Pyelonephritis

Electrophoretic analyses of urine from patients with pyelonephritis, acute as well as chronic, gave patterns (figs. 6 and 7) which as a rule differed essentially from those discussed above. The curve for the albumin fraction was in most cases high and rather pointed, while the globulin fraction was seen to be considerably larger than in the other forms of proteinuria investigated. Like normal urine that from patients with pyelone

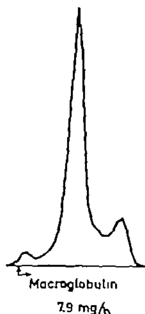


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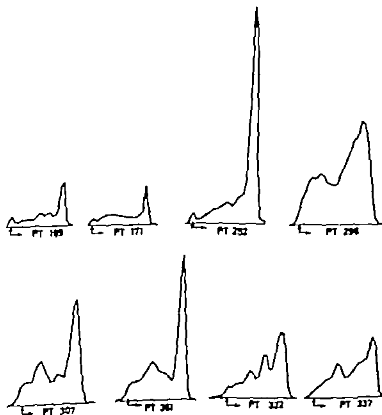


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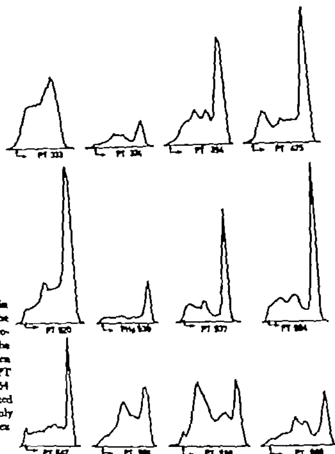


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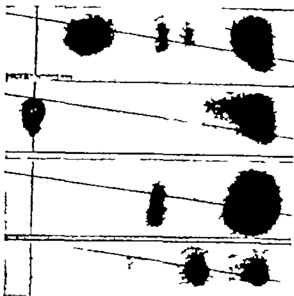


Fig 8. Electrophoretic strips from normal serum (uppermost) and for normal urine (at the starting point a spot is seen consisting of denatured protein, which has been unable to migrate in the electric field) The third strip is from urin in glomerulonephritis and the lowermost from urine in pyelonephritis. The difference between glomerulonephritis and pyelonephritis is here plainly seen.

phritis was found to contain approximately equal amounts of albumin and globulin Unlike normal urine, that from most cases of pyelonephritis shows a marked division of the globulin fraction into α β- and γ-globulin with in particular a γ-fraction definitely larger than normal.

The appearances of the electrophoretic patterns in pyelonephritis, like those in glomerulonephritis are independent of the degree of renal failure and the degree of proteinuria. Further the stated patterns represent acute as well as chronic pyelonephritis. In cases of pyelonephritis with slight proteinuria possibly within the normal range, it may however be difficult to distinguish the pattern from those for normal urine.

The patterns described above are only such as result after the stained electrophoretic strip has been cut to pieces, the dye eluted, and the colour read and plotted in a coordinate system. Evaluation of the stained electrophoretic strip prior to the cutting is, however of great importance in assessing the results. Occasionally we find such a narrow coloured zone which even after cutting into 1/2 cm long strips is not distinctly reflected in the curve plotted after elution and colorimetry

Fig 8 illustrates electrophoretic strips representing proteins in normal serum and normal urine and also the urinary protein in glomerulonephritis and in pyelonephritis. A very marked difference in the urinary proteins is seen between patients with glomerulonephritis and patients with pyelonephritis. In addition it is seen that normal urine contains very little of the most slowly moving globulin

Discussion

About 1 700 urine specimens have been analysed, some normal and some pathological. The pattern shown above for pyelonephritis was not seen in any other form of proteinuria It has been suggested previously by other workers that in lupus erythematosus disseminatus the urinary protein pattern resembles that found in pyelonephritis. The present investigations, including four patients with lupus erythematosus, have not borne out this view In these four cases the diagnosis had been verified by renal biopsy Electrophoresis gave in all these patients a urinary protein pattern resembling that found in glomerulonephritis. This was, in fact, to be expected since the renal changes in this disease are of the same character as in glomerulonephritis. One of these patients had a period with urinary tract infection During this period the electro-

phoretic pattern showed an increased γ-fraction a change which disappeared again after the infection had subsided. In patients with diabetes mellitus similar alterations of the γ-fraction have been observed during urinary tract infections. In such cases the alterations likewise disappeared following recovery from the infection, after which the electrophoretic pattern corresponded to those shown in fig 4

The investigated patients with glomerulonephritis and pyelonephritis were chosen on the basis of past histories typical of the diseases and of laboratory analyses. Renal biopsy had been performed in a single case only In some, the diagnosis had been verified later by autopsy and in one by operation.

The results of the present investigations suggest that this simple test, which is not disagreeable to the patient, serves as an aid in the sometimes extremely difficult differential diagnosis of glomerulonephritis from pyelonephritis. The electrophoretic pattern seen in pyelonephritis differs markedly from those found in other forms of proteinuria. By the method employed here it has been impossible so far to find differences between the urinary protein pattern in glomerulonephritis and those obtained in any other renal disease, except pyelonephritis.

Summary

Electrophoretic patterns for urine specimens from patients with glomerulonephritis and patients with pyelonephritis have been compared. There seems to be a marked difference in composition of the protein the urinary protein in pyelonephritis containing much more globulin than that in glomerulonephritis. Analyses for urinary protein in other renal diseases revealed as in glomerulonephritis a very small globulin fraction

Acknowledgement

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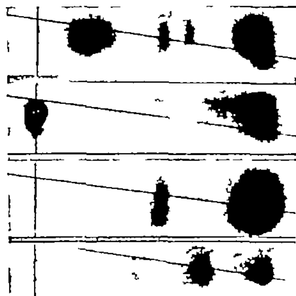


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Aided by a grant from Statens Almindelige Videnskabsfond.

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Analysis of Mortality and Survival in Actively Treated Hypertensive Disease

By

B. Hood S. Björk, R. Sannerstedt and G Angervall

The arrival of potent blood pressure lowering drugs has to some extent changed the picture of hypertensive disease. It is well established that combined drug therapy may prolong survival in malignant hypertension (3 4 6 8, 9 11 12, 13) The findings of the various studies agree surprisingly well.

The longer observation time necessary to establish whether survival is prolonged in other severe varieties of hypertensive disease has produced a delay in information on this point. However Perry and Schroeder (12) and Björk et al. (3) found a decisive increase in survival in such cases. In the latter study all treated patients were classified according to four different systems in order to make possible a comparison with reported survival rates of large untreated series of earlier investigators. Regardless of the system pplied a better survival rate of treated cases was apparent for patients belonging to the two most severe grades (grades 3 and 4) The milder cases (grades 0, 1 and 2) were not included in the study

Submitted for publication March 18, 1963.

Material and methods

The aim of the present study has been an attempt to analyse the mortality of all our patients submitted to treatment with acti hypotensive drugs during the first decade when such drugs were available. The treat ment was started during the period Oct. 1 1950 to Dec. 31 1960, and the observation was terminated on Dec. 31 1961 The patients have been classified according to Keith-Wagener-Barker Palmer Smithwick and Hammarström-Bechgaard. Some cases were not classifiable in all the systems but every case in at least one of them.

An idea of the verage severity of the total material may be obtained from table I wher the numbers of cases in each grade of the respective systems are given. The material includes cases of essential hypertension, chronic pyelonephritis with hypertension as the dominating symptom, and renal parenchymal malformation. Cases of polycystic kidney renal tuberculosis, chronic glomerulonephritis and primary aldosteronism have been ex cluded. In the last four years w ha diag nosed well above one hundred cases of renal artery stenosis. These have also been excluded, as the choice between surgical and drug treatment in renal artery stenosis or a combination of both will be the subject of a

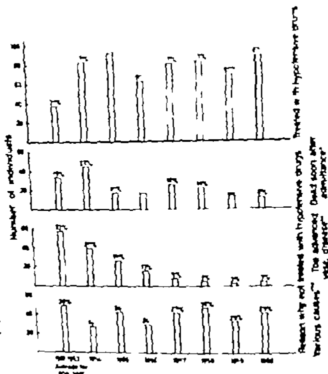


Fig. 1. Successive change in the proportion of hypertensive patients put on treatment with hypotensive drugs. Reasons for refraining from treatment.

x) Patients dead during their first hospital stay — the majority within the first 72 hours due to cerebro-vascular lesion.

xx) Patients with frank uremia, massive cerebro-vascular lesion, fresh myocardial infarction or repeated myocardial infarctions.

xxx) Patients with too mild hypertension (the major part), malignant tumours in advanced stages, psychosis or severe chronic alcoholism.

We have also tried to evaluate whether any change in the mortality pattern has appeared throughout the 10-year period after introduction of active therapy.

The causes of death have been assembled under the following different headings and the respective groups have been separately treated:

a) cerebro-vascular lesions,
b) myocardial infarction,
c) congestive failure,
d) uremia,
e) other causes directly or indirectly related to the hypertensive disease,
f) causes related to the regimen used
g) causes with no relationship to the hypertensive disease.

Results

Total mortality

A retrospective analysis of the first three years of active treatment shows that at that time we had only scratched the surface of the immense problem of treating hypertensive disease. In one of the two participating departments (Gothenburg) where treatment was started 900 patients were admitted with the diagnosis of hypertensive disease during these three years. Five hundred and twenty-eight were below 65 years, but only 21 per cent of them were submitted to active treatment. Nineteen per cent were admitted with a rapidly fatal complication. Thirty-two per cent were considered to have too severe vascular disease to be treated (fresh myocardial infarction, frank uremia, fresh severe cerebro-vascular lesion). Twenty-eight per cent were not treated for various other reasons: the majority because they were considered to have too mild hypertensive disease, some because of severe chronic alcoholism or malignant tumours.

Table I Distribution of actively treated patients in the different classification systems

Grade	Keith-Wagener-Barker			Palmer			Smithwick			Hammarström-Bechgaard		
	Living	Dead	Survival (%)	Living	Dead	Survival (%)	Living	Dead	Survival (%)	Living	Dead	Survival (%)
IV	57	57	50	57	57	50	106	89	54	113	93	54
III	95	63	60	393	139	74	186	76	71	364	94	79
II	384	71	85	205	17	92	329	49	87	178	13	93
I	183	23	89	96	3	97	105	2	98	71	2	97
0	39	1	98	1	—	100	2	—	100	—	—	—
Unclassifiable	14	3	82	20	[illegible]	90	44	2	96	46	11	81

These figures include all patients below 66 years of age at initiation of treatment. Hence they cannot be used directly for comparison with Hammarström Bechgaard's survival curves, which are founded on individuals below the age of 60.

special study. We are, however, fully aware that the material of the present investigation must contain a number of cases from earlier years with undiagnosed renal artery lesions.

During the period covered by this study 990 cases initially examined as inpatients have been submitted to active treatment. A limited number of patients in whom the clinical data were adequate for classification of the initial status has been included, although treatment was started some time before admission on an outpatient basis. During the first 3-year period we refrained from treating patients with advanced uremia, fresh massive cerebrovascular lesions or fresh massive myocardial infarction. During the latter years all cases of uremia but subagonal have been included. Also patients with seemingly massive cerebrovascular lesions presenting high blood pressure levels have been treated. To be included in the study we have insisted that the cases surviving the first hospital stay should be known to have made at least two visits to our outpatient department or to other physicians after leaving the hospital.

As discussed in earlier papers we think that our results are of interest from one special point of view. It is a large series of cases and still it has been handled within the ordinary activities of the two university departments participating. No special facilities such as hypertensive clinics have been set up. This has certainly increased the number of patients disappearing from treatment and thus the number of failures. However, the often expressed wish that the severe varieties of hypertensive disease should be managed by specialists in hypertension exclusively is not practical policy in our way of thinking. The number of specialists required for this and other important fields is prohibitive. We believe that the obligation of rendering first class care for one of the most frequent diseases and causes of death within the field of internal medicine rests firmly with the specialist in this field. The outpatient clinic, specializing in one disease is to us a research tool. The advantages, drawbacks and difficulties of our way of handling the material should probably be more representative of the problems appearing in all large, well-equipped and well-staffed medical departments.

All the records of the patients who have died (218 cases) have been carefully worked through by two members of the team. Doing this we have especially tried to arrive at a conclusion as to whether death might be ascribed to initial severity of the hypertensive disease or to an insufficient degree of control of blood pressure. Where control was inadequate, we have tried to establish whether this was due to conscious planning in the presence of severe vascular disease, to failure of the doctor in charge to plan, inform and instruct, or to failure of the patient to cooperate and if so the reason for this.

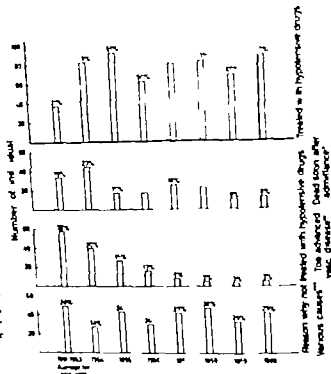


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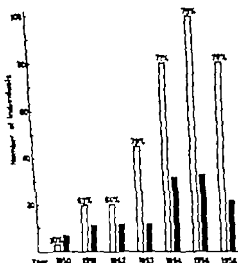


Fig. 3. 5-year survival and mortality of patients whose active treatment started in the years given on the abscissa. Patients surviving 5 years. ■ Patients dead within 5 years.

When instead the five-year survival is calculated for the patients where this was possible, i. e. those starting the treatment from 1950 to and including 1956 no very great change is found to have occurred even if some increase is seen (fig. 3). For those starting treatment in 1953—1956 the five year survival period is practically the same and lies around 70—80 per cent. However this finding has to be viewed against the above reported decreasing figures of mortality soon after admission of untreated patients, as well as the decreasing number of patients not treated because of too advanced vascular disease.

Causes of death

The number of individuals who have survived for a certain time after the beginning of treatment and died of the major causes of death, uremia, cerebro-vascular lesion and myocardial infarction, has been calculated (fig. 4).

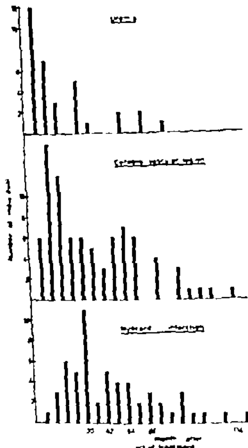


Fig. 4. Duration of active treatment before death in cases dying of uremia, cerebro-vascular lesion or myocardial infarction.

As might be expected an accumulation of deaths from uremia is found within the first 12 months after initiation of treatment caused by advanced malignant hypertension. A peak of mortality from cerebro-vascular lesion occurs within 12—18 months comprising mostly cases which were never brought under good control. In contrast no such sharp mortality peak is seen in the group of lethal myocardial infarctions. Here the deaths seem to be more evenly distributed throughout the years.

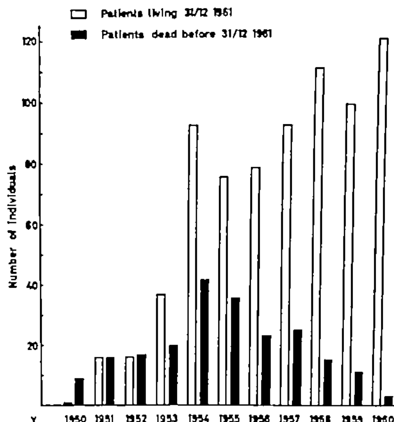


Fig 2 Total material (990 patients) arranged according to the year when active treatment started.

There was a marked increase in the percentage of patients in hospital (Gothenburg) submitted to active treatment in the course of the years (fig 1) A decrease in the figures for acute deaths among untreated patients as the years go by is clearly seen but due to a reorganization of the hospital the figures for 1959 and 1960 may be somewhat too low The absolute and percentage numbers of cases not submitted to treatment because of too advanced vascular disease show a remarkable successive decrease. During recent years attempts at treatment have been made even in massive cerebro-vascular lesions and manifest uremia if diastolic levels were high. However there seems to be no doubt that a substantial decrease in the number of cases admitted in such an acute state has occurred At any rate it may be safely stated that treatment has not been withheld due to the severity of the disease with the exception of a hand ful of cases in so bad a condition that treatment was considered to be absolutely meaningless.

The proportion of patients not treated due to various other reasons among which too mild a disease plays the dominant role, has been surprisingly constant throughout all the years.

One gets the impression that there is a steadily rising proportion of survivors as compared with fatal cases when the patients are arranged according to the initial year of active treatment (fig 2) Coupled with the fact that fewer and fewer cases were excluded from treatment because of too severe vascular disease (fig 1) this might seem very striking but the figures are, of course, invalidated to a large extent by the short observation time of the patients beginning treatment during more recent years.

where the degree of vascular disease — i. e. uremia or severe cerebral deterioration forced the physician in charge to withdraw treatment successively — five cases.

b) Those who had had more or less consistent therapy but who had suddenly dropped treatment a short period before death (from 24 hours to 2 months, usually 1—2 weeks). This group contained 16 cases. Five of these were themselves responsible for the withdrawal due to misinterpretation of instructions, or simply forgetting the drugs when going on a journey. In 11 cases other physicians stopped the treatment abruptly usually because of more or less severe side effects of the drugs. In a few cases, however the reason had apparently been given that the blood pressure level was so good that no cumbersome treatment was required". This interference from other physicians not responsible for the induction and maintenance of active treatment has become more rare during recent years. However according to reports from a small number of patients living under continuous and good control, who have successfully withstood such seductive influences, it still occurs now and then.

In 6 cases it could not be ascertained whether the patients adhered to therapy until death — although in each of them lack of regular visits for a very long time made this highly improbable.

Nature of the cerebro-vascular lesion and degree of control. Of the 84 patients dying of cerebro-vascular lesion one had a large fibrillating heart and developed multiple cerebral emboli. Fifty-four of the 84 were autopsied. Seventeen of these proved to have cerebral infarction with malacia, the rest had massive cerebral hemorrhage. The serious lack of control for the whole group has been discussed above, but the question remains whether sudden severe blood pressure lowering due to the drugs might have precipitated cerebral infarction.

Of the 17 cases dying of cerebral infarction 13 had no therapy at the time of the attack. In one case it was unknown whether the patient had adhered to therapy or not. Three had moderate blood pressure reduction at their last control visit. In none was there any marked reduction. In one of the 3 with moderate control there was a thrombosis in one carotid artery with complete obstruction. One further patient (early case, 1951) with severe malignant hypertension treated with hexamethonium subcutaneously had had several severe postural reactions and in this case the possibility has to be considered that these might have been of importance.

Myocardial infarction

The peak of mortality in myocardial infarction appeared at 30 months after the initiation of treatment and mean survival time was also 30 months (fig. 4).

To the group of myocardial infarction we have referred all sudden deaths not otherwise explainable, with full recognition of the uncertainty this introduces. Twenty-two of these patients had suffered myocardial infarctions or severe angina pectoris before or during treatment. Autopsy was obtained in only 31 patients.

More than half of the patients, 30 out of 53 were under continuous treatment with significant reduction of the blood pressure at the time of death (table II). In two cases the reduction was registered as not significant, in 13 as moderate and in 15 cases or close to one third of the whole material the degree of control was estimated as excellent.

Table II State of therapy at death from the four major causes

	Continuous therapy B. P control			Continuous therapy abandoned 1—60 days before death		Therapy abandoned ≥ 60 days before death			Long lasting breaks in therapy	U known	Total
	Marked	Moderate	Not significant	Physician's responsibility	Patient's responsibility	Too severe vascular disease	Physician's responsibility	Patient's responsibility			
Cerebro-vascular lesion	2	16	9	11	5	5	5	20	4	6	83
Myocardial infarction	15	13	2	1	2	5	2	11	—	2	53
Congestive failur	—	2	2	—	—	5	3	1	—	—	13
Uremia	3	4	5	—	—	10	3	8	—	—	33

One case of repeated cerebral embolism from a fibrillating heart has been excluded in this tabl

The deaths due to sudden vascular catastrophes (cerebro-vascular lesions, 84 cases, and myocardial infarctions, 53 cases) were by far most frequent.

The degree of efficiency of therapy at the time of death as well as the causes for abandoning therapy for cerebral vascular lesions and myocardial infarctions and for the four major causes of death together have been given in table II

Cerebro-vascular lesions

The peak of mortality in cerebro-vascular lesions occurred between 12 and 18 months after the initiation of treatment and the mean survival time was close to 24 months (fig 4)

The group contains a striking collection of therapeutic failures (table II) Approximately half of the patients (40 of 84) had had evidence of cerebro-vascular disease before induction of therapy — most of them in the form of acute cerebro-vascular lesions, of which about half had left permanent sequelae. A few cases were included in this group as the showed severe cerebral deterioration i the absence of features of malignan hypertension Less than one third of th patients (27) had been under continuou therapy and in only 18 of these cases significant reduction of blood pressur was considered to have been achieved In only two cases was the reduction of blood pressure considered as marked — i e. consistently more than 35 mm Hg diastolic Four patients had reinstitution of treatment with moderate success during their final months, but had previously dropped treatment for periods of up to several years. In each of these four cases the time without drugs constituted the major part of the period of observation.

The 46 patients who were known to have abandoned treatment altogether might be divided in two fairly sharply delineated groups

a) Those with whom the treatment slowly deteriorated and where patients stayed away from control — 25 cases or

where the degree of vascular disease — i. e. uremia or severe cerebral deterioration forced the physician in charge to withdraw treatment successively — five cases.

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uremia. Nine patients showed a rapid loss of renal function, 13 patients showed a slowly progressive renal failure with a survival from 14 to 60 months and a mean survival of 27 months. In 7 of these control was considered good. It was maintained that this delayed renal failure showed no close correlation to the level of supine diastolic pressure. The authors found a diffuse fibrous intimal hyperplasia that sometimes appeared occlusive in larger renal branches (arcuates and interlobar arteries) This lesion was believed to develop in spite of good pressure control.

Our experience differs from that of these authors.

Of our 33 patients dying of uremia (fig 4 and table II) 25 had eyeground changes characteristic of malignant hypertension. In 15 of the 33 the serum creatinine was more than 3 mg % initially Of the remaining 18 the majority had moderate renal impairment.

Of these 18 patients 9 were from the first year when active treatment was in its infancy (1950—1951) and they were treated with peroral hexamethonium only with little or no change in their rapidly deteriorating condition.

Dustan et al. (4) used 14 months to delineate what they called delayed renal failure Six patients in our non-uremic group survived for more than 14 months, living 26, 27 29 52, 62 and 74 months respectively Three of them escaped from control after only few months. One of these three cases had also chronic pyelonephritis.

A young male who died after 26 months in uremia was living at a long distance from the department and, in spite of a rather harsh three-drug program, presented lack of control with diastolic levels between 130 and 150 mm Hg

One girl of 17 years with bilateral renal malformation had excellent control for three and a half years. Her serum creatinine level was unchanged or even somewhat improved She then became pregnant against ad ice, stayed away and went completely out of control. A super imposed severe toxemia was treated with drastic measures and she gave birth to a living healthy child although in renal failure Soon after that she died in progressive uremia.

One male of 50 years lived during 74 months under excellent control However he had from the outset slight anemia and sedimentation rates around 100 mm/h, a slight pyuria and coliuria. His autopsy showed severe interstitial infiltration of inflammatory cells with abscess-like formations in the renal cortex. The major renal arteries showed no obstructive lesions.

In the group with initial serum creatinine levels above 3 mg % 5 patients survived 14 months. Three of them proved to have chronic phenacetin abuse and latent chronic pyelonephritis with characteristic microscopical changes. One of these had fundal changes of Keith-Wagener s grade 3 the other two only hypertensive angiopathy The remaining cases both had severe lack of control

A large number of cases w th severe initial derangements of renal function (> 3 mg % serum creatinine nitially) have survived for periods of up to 3 years under partial control with a static renal function and have died in cerebrovascular hemorrhage. Thus, not one of all our patients dying of uremia who had renal vascular changes alone at the outset progressed to death under good pressure control. There was always either parenchymatous renal disease or lack of control or both.

No patients had made long lasting breaks of treatment and then had it readjusted — as had happened in four cases of the cerebro-vascular lesion group

The group where there had been more or less successful therapy and then a sudden withdrawal a short time before death contained only 3 patients as compared with 16 in the group of cerebro-vascular lesions. In the group of 18 patients where treatment had been abandoned a long time before death the patient was considered to be responsible for this in 11 cases. In 6 patients angina pectoris became apparent during treatment and was the cause of the withdrawal of active treatment.

In this group there were only two patients whose treatment had been stopped by a physician for reasons that we consider might not have been imperious as far as it is possible to judge retrospectively

The problem whether the induction of low pressure may precipitate myocardial infarction and how frequent such an occurrence might be seems to be a difficult one. In our experience cases admitted with myocardial infarction during treatment usually present with rather high blood pressure levels, decreasing when pain has been controlled. During the initiation of active drug regimen hypotension is probably more frequent than later However only one of our 53 patients died within the first 6 months after the induction of such treatment (fig 4) On the other hand in three cases, where the reduction of blood pressure was known to have been very marked from very high levels to normotension or near normotension death occurred during night. These cases have tentatively been considered as precipitated by drug induced hypotension.

Congestive failure

Thirteen patients have been recorded as dying from congestive failure. Nine of these had dropped the treatment for periods varying from 2 to 33 months before death. Of the four patients on active treatment up to the time of death, three had shown severe congestive failure at the outset. Two of these patients died suddenly They had extensive coronary sclerosis but no occlusive lesions and no signs of fresh myocardial infarction. The third patient, a woman of 54 years, arrived in malignant hypertension with extreme congestive failure. The therapy during the first six months was adequate with marked blood pressure reduction and improvement of congestive failure. She then developed severe edema of the lower arms. Hydralazine was dropped and the edema slowly subsided Afterwards the blood pressure control was lost in spite of consistent efforts. She died after 12 months. The fourth case was a woman of 39 years with eyegrounds of Keith-Wagener's grade 3 and no symptoms from the heart at the outset. She rapidly deteriorated and was during the whole course totally resistant to the drugs (reserpine, pentolinium hydralazine) Congestive failure appeared and she died dramatically of functional aortic incompetence. Initially adreno-cortical disturbance was suspected and looked for but could not be verified clinically This was in 1953 The autopsy revealed however an adrenal cortical adenoma the size of a walnut.

Uremia

Page's group (Dustan et al (4)) in their analysis of a large series of cases of malignant hypertension had 22 deaths in

pentolinium. Due to a series of unfortunate circumstances, she was treated too late and insufficiently with norepinephrine.

One patient apparently died in hypopotassemia. This patient had for several years been fully active and in good control on a therapy with 750 mg chlorothiazide daily and a somewhat varying dose of pentolinium. During the last year he had suffered somewhat from thirst and polyuria. Six weeks before death he had diarrhea for two days after which he developed stiffness in the muscles of the neck and arms. Three days before admission he had been vomiting after every meal. On admission he showed acute respiratory distress with involvement of the auxillary respiratory muscles. In spite of immediate treatment with infusion of potassium salts he died within five hours. Serum potassium proved to be 1.7 mEq/l. Autopsy showed no changes in adrenals. The kidney tubuli showed signs of vacuolization.

Causes of death having no relationship to the hypertensive disease

In 10 patients death was considered to have no relationship to the hypertensive disease (table IV).

Only one of the cases requires comment. This was a patient who had shown signs of acute cerebro-vascular lesion. When therapy was introduced he had eyegrounds of Keith-Wagener's grade 1. This patient was controlled at a local hospital at some distance from our department. Three years later an acoustic tumour was discovered. Medicamental treatment was dropped and he died apparently because of the tumour. There is nothing definite to indicate that symptoms caused by his hypertension delayed the correct diagnosis.

Table IV. Mortality with no relation to the hypertensive disease

	No. of cases
Adenocarcinoma	
Cancer of the stomach	1
Cancer of the colon	4
Cancer of the pancreas	2
Cancer of the lung	2
Cancer of the ovaries	1
Carcinomatosis	2
Hypernephroma	1
Cerebral tumour	1
Carbon monoxide poisoning, accidental	1
Homocide	1
Mitral stenosis, auricular fibrillation, cerebral embolism and bronchopneumonia	1
Ulcerative endocarditis	1
Pulmonary sarcoidosis + influenza	1

Mortality in patients with "mild" hypertensive disease

Twenty-nine of our dead patients did not reach grade 3 in the two classification systems with the strictest requirements for a high grading, i. e. Keith-Wagener-Barker's and Smithwick's systems. One died of uremia, 12 of myocardial infarction and 16 of cerebro-vascular lesion.

The patient dying of uremia had progressive chronic pyelonephritis. The renal work-up before treatment was unsatisfactory. Some of the laboratory tests would possibly have placed her as belonging to Smithwick's grade 4. However, in doubtful cases we have (as mentioned in earlier papers) always given precedence to a lower grading.

Of the 12 patients dying of myocardial infarction only 4 had treatment up to the time of death. In 2 of these, the blood pressure control was consistently good, in the other two probably not significant. In a fifth patient pentolinium was stopped one week before death. Seven had a-

Table III Causes of death related to the regimen used

Sex	Age of death	Adequacy of treatment	Dead months after start of treatment	Degree of B. P control	Cause of death
♀	55	Adequat	5	Moderate	Cerebral edema
♀	37	Adequate	26	Not significant	"Bizarre dyspnea
♂	61	Adequate	43	Moderate	Cardiosclerosis + chronic pneumonitis
♂	58	Adequate	12	Marked	Suicide (Reserpine)
♀	54	Adequate	16	Moderate	Pentolinium poisoning
♂	58	Adequate	52	Marked	Hypopotassemia Respiratory failure

Other causes of death directly or indirectly related to the hypertensive disease

In 10 further patients we think that the death had a direct or indirect relationship to the hypertensive disease.

Three patients (aged 41 55 and 56) died of dissecting aortic aneurysm. One of them had his lethal attack 17 months after having abandoned the treatment. In another there was a continuous moderate degree of control In the third the control was wholly insufficient throughout the course due to stenocardiac symptoms. Six patients died of bronchopneumonia one patient died of gastric hemorrhage We have interpreted these deaths as having some relationship to the hypertensive disease, since our experience is that cases of this type would not die in a hospital with the modern treatment available, if there were no serious condition in the background

Causes of death related to the regimen used

In 5 patients we have considered the death in relation to the regimen used (table III)

Cerebral edema was the cause of death in a woman of 55 years admitted in a suburemic malignant state with vomiting Treatment was induced with a large dosage of chlorpromazine and an extremely small dose of parenteral pentolinium simultaneously The blood pressure was reduced to normal. In the belief that pentolinium in the dosage used (fractions of 1 mg) was without importance, this drug was suddenly withdrawn. Within 30 hours there was an acute rise of blood pressure and a fulminant cerebral edema developed with lethal outcome.

One of the first treated malignant cases developed a typical syndrome of bizarre dyspnea after 26 months. This is the only case with a clearcut picture of this syndrome in our material. However in a male of 61 years who died after 43 months of treatment and who was autopsied in a hospital in another town it was noted in the postmortem that he had a chronic pneumonitis.

The only case of suicide in the series must be interpreted as precipitated by reserpine.

A 54-year-old woman on a successful regimen, while at home in a disorientated state during the course of an acute gastroenteritis took a very severe overdose of

others with the group of malignant hypertensives arriving too late in advanced renal failure. Our series also include the early group from 1950 and early part of 1951 on peroral hexamethonium only who progressed under incomplete control.

However our experiences differ most markedly from those of the Cleveland group. We have never seen what they call "delayed uremia". In other words, we have not seen patients under good control progressing from moderate renal impairment to uremia due to fibrous changes in medium-sized renal arteries. We have seen only a few cases progressing in spite of moderate or in one instance, excellent control and they were all patients with phenacetin abuse interstitial nephritis and hypertension.

The two main causes of death, cerebro-vascular lesion and myocardial infarction differ markedly regarding their relationships to therapy.

In the group of myocardial infarctions most patients were under continuous treatment at death and half of them died while under good or excellent control. In contrast to this, the group of cerebro-vascular lesions was to a large extent a collection of therapeutic failures. Only a minority had treatment at all at the time of death, and only one single patient had excellent control until death. In a considerable number of cases control had never been good or deteriorated slowly for various reasons. However in quite a number of patients the cerebro-vascular lesion occurred within a short time after sudden withdrawal of treatment. This sudden withdrawal was most often undertaken on what appeared unjustified advice from other physicians or in some cases due to neglect or misunderstanding on the part of the patient. However there were in this group of cerebro-vascular lesions also cases where the blood pressure due to severe renal impairment or to angina pectoris was impossible to keep at a satisfactory level.

To us it looks as if by increasingly better methods of treatment and by better information to colleagues and patients many cerebro-vascular lesions may be preventable. On the other hand, good or excellent control did not prevent a number of lethal myocardial infarctions. This does not seem more surprising than the deaths occurring in myocardial infarction in normotensives. However it is reasonable that *a reduction of blood pressure* might slow the rate of atherogenesis.

Only very seldom have we found cases where there might be entertained a definite suspicion that too intense blood pressure lowering might have precipitated either myocardial infarction or encephalomalacia.

Summary

1. The number of patients with hypertensive disease admitted dying or with massive vascular complications beyond the limits of reasonable therapy has decreased markedly during the first 10-year period of active treatment.

2. The number of patients not submitted to active treatment due to too mild hypertensive disease, psychosis, chronic alcoholism or malignant tumours seems to be surprisingly constant during this decade.

3. The chance for 5-year survival seems to have gained somewhat from the first years but has from 1953—1956 been stabilized around 70—80 per cent.

bandoned treatment for periods from 6 months up to 5 years

Of the 16 patients dying of cerebro-vascular lesion only 4 had treatment up to the time of death One of the 12 remaining had gone on a vacation two weeks before death without taking the drugs with him. The other 11 had abandoned treatment for periods from 6 months up to 6 years

Of the 4 patients who had treatment until death one patient had had long periods without treatment at all and even on drugs he had widely swinging blood pressure records Three were under consistently good or excellent control. Of these one a male of 43 years had suffered two cerebro-vascular lesions without sequelae before treatment started. Another had earlier been off treatment for a long period but had fairly good control towards the end. The third, a highly nervous woman had severe trouble with the drugs but showed a marked lowering of her initially very high diastolic level (170 mm Hg) She had however never any resemblance of complete control.

Thus, among these 29 patients, who viewed from their grading died somewhat surprisingly cerebro-vascular lesion and myocardial infarction dominated completely as causes of death Only 8 were under continuous treatment until their death and of these only 3 were known to have had real good control throughout their course Of these 3 one dying of a cerebro-vascular lesion had suffered two cerebro-vascular attacks before treatment. The other two died of myocardial infarction. One of these had angina pectoris from the outset. The other had no subjective symptoms, but presented at the autopsy very extensive coronary atherosclerosis and signs of old infarction.

Discussion

The aim of this report has been to concentrate the analysis on the deaths to see whether lessons might be learned for the future. In the beginning of the 1950's more than half the hypertensives below 65 years admitted to hospital in the Gothenburg department were admitted either with their fatal complication (19 %) or with too advanced disease to be considered to benefit from active therapy (32 %) This state of things has changed dramatically over the years. It should be remembered that vast numbers of patients with milder hypertensive disease have at a steadily increasing scale been brought under control in the various out patient departments and in private practice. However active treatment has also been tried more and more often in patients with severe vascular disease.

In the actively treated material the number of survivors are rapidly piling up and the number of deaths lagging However when the analysis is confined to the cases where there are at least 5 years of observation i e. the patients starting treatment from 1950 to the end of 1956 it is seen that there is only a small gain in 5-year survival the figures stabilizing around 70—80 % in the four groups starting treatment 1953 1954 1955 and 1956

The pattern of mortality from the different major causes of death seems to have changed quite distinctly

The formerly leading cause of death, congestive failure has dropped to insignificance. The isolated cases seem to be due to either a combination of advanced vascular disease and extreme congestion before the treatment or in most of the cases to the treatment being abandoned.

In the group dying of uremia we naturally have the same experiences as

others with the group of malignant hypertensives arriving too late in advanced renal failure. Our series also include the early group from 1950 and early part of 1951 on peroral hexamethonium only who progressed under incomplete control.

However our experiences differ most markedly from those of the Cleveland group. We have never seen what they call "delayed uremia" In other words, we have not seen patients under good control progressing from moderate renal impairment to uremia due to fibrous changes in medium-sized renal arteries. We have seen only a few cases progressing in spite of moderate or in one instance excellent control and they were all patients with phenacetin abuse interstitial nephritis and hypertension.

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In the group of myocardial infarctions most patients were under continuous treatment at death and half of them died while under good or excellent control. In contrast to this, the group of cerebrovascular lesions was to a large extent a collection of therapeutic failures. Only a minority had treatment at all at the time of death, and only one single patient had excellent control until death. In a considerable number of cases control had never been good or deteriorated slowly for various reasons. However in quite a number of patients the cerebrovascular lesion occurred within a short time after a sudden withdrawal of treatment. This sudden withdrawal was most often undertaken on what appeared unjustified advice from other physicians or in some cases due to neglect or misunderstanding on the part of the patient. However there were in this group of cerebrovascular lesions also cases where the blood pressure due to severe renal impairment or to angina pectoris was impossible to keep at a satisfactory level

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3 The chance for 5-year survival seems to have gained somewhat from the first years but has from 1953—1956 been stabilized round 70—80 per cent.

4 Congestive failure as a cause of death has dropped to an insignificant position

5 We have not observed any progress from moderate renal impairment into uremia in a single case under good control if primary renal parenchymatous disease was absent

6 Half of the deaths from myocardial infarction occurred while the patient was under good or excellent blood pressure control However while not being able to prevent the myocardial infarction the blood pressure reduction was only in a few instances of such degree that it might be suspected to have precipitated it.

7 In contrast to the myocardial infarction group the cerebro-vascular lesion group on the whole contained a number of therapeutic failures. Only a minority of patients were under good control at the time of death. The group of cerebro-vascular lesions below 65 years should to a large extent be preventable with better instructed physicians and patients. The steady successive improvement of drugs should also contribute to this.

8. We have collected a group of what we call "surprisingly dead in other words patients dying although the gradings in the various classification systems were rather low These patients died either of cerebro-vascular lesion or of myocardial infarction. When submitted to a close analysis, the reasoning for these two groups is in good correspondence with that given above in 6 and 7

Acknowledgements

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Macroglobulinemia or Multiple Myeloma

By

Knud Kjeldsen and Vivian Harding Asfeldt

Macroglobulins are normally present in human sera, in very low concentration (approx. 2 % of the total protein). In pathological conditions there may be an increase in the concentration of macroglobulin. Macroglobulinemias are normally classified as primary (Waldenström disease) or secondary the latter of which can be seen in a number of various diseases especially neoplasms, collagen diseases and chronic infections (26).

Originally it was presumed that the non-physiologic macroglobulins were abnormal proteins (paraproteins) but later work seems to suggest that they are normal globulins in abnormal concentrations (9 16 11).

Osserman (24) defines primary macroglobulinemia as a neoplastic, proliferative disorder of lymphocytic and/or plasmacytic origin, probably related to lymphatic leukemia, lymphosarcoma and myeloma. The disease is seen mainly in elderly men and is characterized by general lassitude, loss of weight, hemorrhagic diathesis, changes in the retina, lymphadenopathy and occasionally spleno-hepatomegaly. Examination of the blood reveals hyperglobulinemia and very high ESR. Electrophoretic examination of the serum shows a considerably increased β- or γ-globulin fraction whilst immunological investigation shows a characteristic change in the β_2-M fraction. Ultracentrifugal investigations show as a rule one or several components with a high molecular weight (19—20 S). The bone marrow often shows a characteristic cytology with increased numbers of lymphoid cells, but often also an increase in the reticulum cells, plasma cells and lymphocytes.

The etiology is unkown. Possibly it is a hereditary disease (21). Chromosome changes are described (4). No curative treatment is known.

Primary macroglobulinemia was described for the first time in 1944 (34). There have since been published more than 100 cases (12). Detailed descriptions are given by Schulten and Kanzow (30)

Submitted for publication March 25, 1963

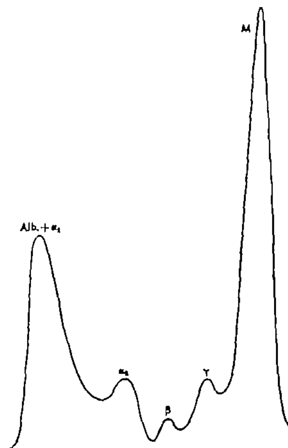


Fig 1 Free electrophoresis a.m. Antweiler Total protein 7 9 g/100 ml. Albumin + α_1-globulin 34.5 α_2-globulin 8.8 β-globulin 1 7 γ-globulin 7 1 % M-component 47 9 %

Waldenström (37) Kappeler et al (15) and Ritzman et al. (26)

The majority of authors consider primary macroglobulinemia as a well defined clinical state which can usually be easily distinguished from multiple myeloma and lymphatic leukemia (34 40 36 19 12 18, 25) Several authors (6 14 33 41) have described cases of "atypical macroglobulinemia which they considered were transitional forms between multiple myeloma and primary macroglobulinemia. Heremans and Heremans (11) and Imhof and Ballieux (13) have recently examined 40 such cases and shown that all these cases were β A myeloma. In none of the cases was the sedimentation constant higher than 12 S. Actual transitional forms are rare, but can be seen (2 23 38 39) Only in 2 of these cases has β_2 M macroglobulin (2 39) been demonstrated. The presence of both γ-myeloma protein and β M macroglobulin in the same patient does not appear to have been previously described in literature.

Case report

An 81 year-old man recurrent attacks of arthritis urica since 1943 myocardial thrombosis in 1961 and symptoms of prostate hypertrophy for several years. Transferred on the 11 7 1962 from another hospital where he had been admitted complaining of general lassitude and loss of weight. On admission the patient appeared psychologically normal, pale, emaciated, and the only objective symptom appeared to be a quite benign prostate hypertrophy There were no findings of palpable lymph glands, enlargement of the liver or spleen, bone tenderness, bone protrusions, Raynaud-phenomena or hemorrhage n the epidermis or mucus membranes.

During hospitalization the disease was characterized by numerous periods of diarrhea with bloody stools. The diarrhea resulted in severe anemia and definite changes in the serum electrolytes. Despite intensive treatment with blood transfusions and electrolyte infusions the patient gradually deteriorated. Finally the patient developed pneumonia and died on the 5 8.62

Laboratory tests

Blood. WR 21 KR 18, MR strong TPI (Nelson) indeterminable owing to anticomplementary serum. Hemoglobulin 7?—59 ESR 146—112 mm/hour Erythrocytes 3.30 mill./mm³ Leukocytes 4,280 /mm³ Color index 0 99 The differential count showed 17 juvenile neutrophils, 55 segmented neutrophils and 28 lymphocytes of normal appearance. The erythrocytes showed definite signs of rouleau formation.

Blood chemistry Serum sodium 135—131 mEq/l Serum potassium 4.9—5.7 mEq/l

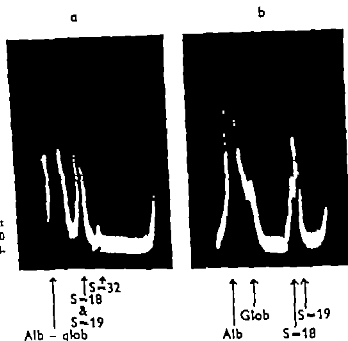


Fig. 2. Ultracentrifugation at 1,000 RPS. Exposures after 10 min (a) and 30 min (b). Sedimentation from left to right.

Serum chloride 106—92 mEq/l. Total CO 16.8—20.6 mEq/l. Serum calcium 4.6 mEq/l. Inorganic serum phosphate 6.0 mg/100 ml.

Urine: Varying amounts of protein, max. 0.3 g/24 hours. There was no Bence-Jones protein. Electrophoretic investigation was not carried out. Microscopic examination of the urine sediment was normal.

Feces: Occult blood test strongly positive. No pathological bacteria from the alimentary canal could be demonstrated on repeated bacteriological investigations.

Protein tests: Serum protein 7.9—5.5 g/100 ml. On electrophoretic examination of the serum (... m. Antweiler) an M-component in the γ-position was found which comprised 47.9 ... of the total protein (fig. 1).

An ultracentrifugal test was carried out on serum diluted to 1 : 4 with 0.2 ... NaCl. This showed (see fig. 2) that 25.2 % of the protein had ... sedimentation coefficient of ... = 13.8 S, 12.1 ... had ... = 15.3 S and 1.5 ... had ... = 31.3 S, such that all together 36.8 % of the serum protein probably must be considered as being macroglobulins. On account of this high macroglobulin content, the tests were repeated after additional dilution with 0.2 n NaCl to 1 ... 8 and 1 ... 16 of the original concentration. The s-values derived from these must be considered as being more accurate and on extrapolation to zero concentration, where the most ideal conditions for unimpeded sedimentation appear, the sedimentation coefficient for the first two components was calculated to be ... = 18 S and ... = 19 S.

The overall evaluation of the ultracentrifugation would suggest primary macroglobulinemia, although it is unusual to find a division of the macroglobulin peak as seen here (carried out ... the Carlsberg Bryggeriernes Forsøgslaboratorium, sgn. R. Djurtoft).

On immunoelectrophoresis 2 M-components were shown: ... γ-M component with slow migration speed and ... β_2-M component. The amount of the fast γ-globulins was normal, whilst the β_2-A globulin, the siderophilin and the albumin were reduced, and ... α_2-haptoglobin was increased. The migration speed of the albumin was slightly higher than normal (carried out on Københavns Universitets Biokemiske Institut, sign. J. Clausen) (fig. 3). Sia ... test for euglobulin: a drop of serum produced in distilled water ... heavy precipitate. Cryoglobulin test: positive. Th

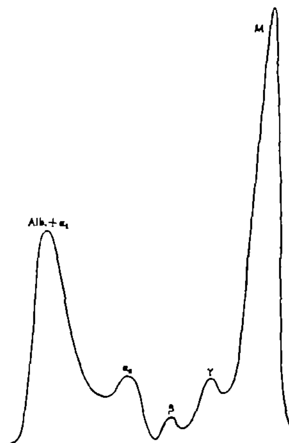


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Blood chemistry Serum sodium 135—131 mEq/l Serum potassium 4.9—5.7 mEq/l

the esophagus, stomach and colon with contrast showed normal conditions.

Eye examination. A slight arteriosclerosis of the retina was seen, but no "fundus paraproteinemicus".

Other examinations. ECG: Bundle branch block P—Q 0.21 sec. Alkaline serum phosphatase 21.0—12.7 units. Acid serum phosphatase 1.1 units. Serum creatinine 6.9—4.5 mg/100 ml. Serum bilirubin 0.3 mg/100 ml.

Post-mortem examination. The most important findings were: left-sided croupous pneumonia with a fibrinous pleurisy, hypertrophy of the heart with diffuse myocardial fibrosis and arteriosclerosis of the coronary arteries, hypertrophy of the prostate and chronic pyelonephritis. There were no focal changes in the columna.

The histological examination showed myocardial fibrosis, pyelonephritis chronica and stasis of liver and spleen. There was found slight hyperplasia of the red bone marrow but no plasma cell increase and no foci of plasma cells. Tissue was taken for microscopic examination from the heart, tongue, suprarenal glands, spleen, liver, kidney, striped muscle and bone marrow, but in no case was it possible to show PAS-positive homogeneous infiltrations (sign. G. Hjort).

Discussion

The case history is naturally difficult to evaluate, as both primary macroglobulinemia and myelomatosis have a number of similar symptoms. Either disease could thus be responsible for the patient's loss of weight, anemia, high ESR, tendency to hemorrhage, increased thymol extinction, cryoglobulinemia, positive Sia test and proteinuria. This also holds for the positive WR and the inconclusive TPI (35). Whereas the lack of the important symptoms, such as bone pains and osteolytic lesions of the bone, disfavors myelomatosis, the lack of lymph gland tumor, spleno-hepatomegalia and retina changes disfavors macroglobulinemia.

The inorganic serum phosphate was slightly increased and the serum phosphate was initially rather high, but later became normal. On X-ray examination of the columna a slight diffuse halisteresis could be seen. No definite explanation of these findings can readily be given, but it appears most natural to consider them as being the effects of the numerous diarrheas. The protein excretion in the urine was slight (maximum 0.3 g daily); thus it is surprising that the initial high serum protein gradually fell to subnormal values. There is therefore the possibility that the patient also had a protein-losing enteropathy. An examination of this was excluded by the bad general condition of the patient.

The picture was additionally complicated in that the patient also had a chronic pyelonephritis with uremia, which could also have accounted for the anemia, proteinuria and the high ESR, and could also explain the bloody stools. In addition, as M-components can be demonstrated for many years without giving clinical symptoms (11, 39), there is also the possibility that the actual cause of the quick fatal outcome was the patient's chronic nephropathia.

The slight lymphocytosis and reticulosis in the bone marrow support the diagnosis of primary macroglobulinemia. The cells, however, were of normal appearance and did not contain inclusion bodies. PAS-positive homogeneous infiltrations in the organs could not be shown. The bone marrow included only a few plasma cells, the appearance of which was normal, and there was shown no focus containing plasma cells.

Bjørneboe and Gormsen (3) classical studies suggested the possibility of the plasma cells forming antibodies. Later

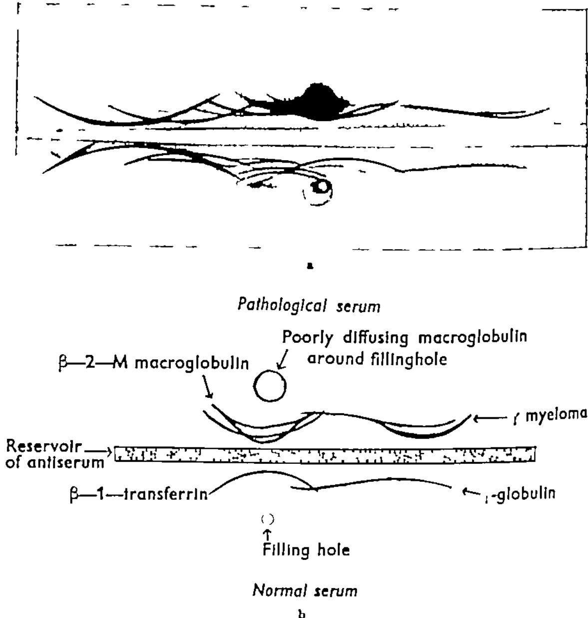


Fig 3 a) Immunoelectrophoresis a.m. Scheidegger (Heremans, Clausen and Rask-Nielsen modification) Pasteur horse anti-human serum ASP 15542 b) Drawing of the most important pathological findings.

kata Ara negative. Thymol extinction 0 50 Prothrombin-proconvertin time a.m. Owren 47 % Bleeding time, hexose content and viscosimetry were not carried out.

Sternal marrow Cell content 22 000/mm³ With the differential count there were found 21 lymphocytes and 6 % reticulum cells. A few of both the lymphocytes and the reticulum cells contained small vacuoles in the cytoplasm, but had on the whole a quite normal appearance. Worthy of note was the fact that there were no inclusion bodies. The other cell systems were normal. There were only 2 plasma cells of normal appearance (sign. H Gormsen)

X-ray examinations No osteolytic lesions of the cranium, costa, pelvis or the long bones. On examination the columna thoracalis and lumbalis showed spondylosis, scoliosis, and slightly diffuse halisteresis. Examination of

the esophagus, stomach and colon with contrast showed normal conditions.

Eye examination. A light arteriosclerosis of the retina was seen, but no "fundus paraproteinemicus".

Other examinations: ECG: Bundle branch block P—Q 0.21 sec. Alkaline serum phosphatase 21.0—12.7 units. Acid serum phosphatase 1.1 units. Serum creatinine 6.9—4.5 mg/100 ml. Serum bilirubin 0.3 mg/100 ml.

Post-mortem examination: The most important findings were a left-sided croupous pneumonia with fibrinous pleurisy, hypertrophy of the heart with diffuse myocardial fibrosis and arteriosclerosis of the coronary arteries, hypertrophy of the prostate and chronic pyelonephritis. There were no focal changes in the columna.

The histological examination showed myocardial fibrosis, pyelonephritis chronica and stasis of liver and spleen. There was found slight hyperplasia of the red bone marrow but no plasma cell increase and no foci of plasma cells. Tissue was taken for microscopic examination from the heart, tongue, suprarenal glands, spleen, liver, kidney, striped muscle and bone marrow but in no case was it possible to show PAS-positive homogeneous infiltrations (mgn. O. Hjort).

Discussion

The case history is naturally difficult to evaluate, as both primary macroglobulinemia and myelomatosis have a number of similar symptoms. Either disease could thus be responsible for the patient's loss of weight, anemia, high ESR, tendency to hemorrhage, increased thymol extinction, cryoglobulinemia, positive Sia test and proteinuria. This also holds for the positive WR and the inconclusive TPI (35). Whereas the lack of the important symptoms, such as bone pains and osteolytic lesions of the bone, disfavors myelomatosis, the lack of lymph gland tumor, spleno-hepatomegalia and retina changes disfavors macroglobulinemia.

The inorganic serum phosphate was slightly increased and the serum phosphate was initially rather high, but later became normal. On X-ray examination of the columna a slight diffuse halisteresis could be seen. No definite explanation of these findings can readily be given, but it appears most natural to consider them as being the effects of the numerous diarrheas. The protein excretion in the urine was slight (maximum 0.3 g daily), thus it is surprising that the initial high serum protein gradually fell to subnormal values. There is therefore the possibility that the patient also had a protein-losing enteropathy. An examination of this was excluded by the bad general condition of the patient.

The picture was additionally complicated in that the patient also had a chronic pyelonephritis with uremia, which could also have accounted for the anemia, proteinuria and the high ESR, and could also explain the bloody stools. In addition, as M-components can be demonstrated for many years without giving clinical symptoms (11, 39), there is also the possibility that the actual cause of the quick fatal outcome was the patient's chronic nephropathia.

The slight lymphocytosis and reticulosis in the bone marrow support the diagnosis of primary macroglobulinemia. The cells, however, were of normal appearance and did not contain inclusion bodies. PAS-positive homogeneous infiltrations in the organs could not be shown. The bone marrow included only a few plasma cells, the appearance of which was normal, and there was shown no focus containing plasma cells.

Bjorneboe and Gormsen (5) classical studies suggested the possibility of the plasma cells forming antibodies. Later

works have with help of isotope techniques been able to show that myeloma proteins are formed in plasma cells (22, 32) That the formation of macroglobulins takes place in lymphoid cells is also well documented (1)

The cytologic picture with primary macroglobulinemia is sometimes dom inated by plasma cells to such a degree that the condition is assumed to be multiple myeloma (8 20 28) It is natural therefore to assume that the macroglobulin in such cases is produced in the plasma cells. Later studies have shown that plasma cells can also form macroglobulins (7) In our case both the macroglobulin and the myeloma protein have probably been produced in the lymphoid cells Other authors have also suggested that with the transitional forms between multiple myeloma and primary macroglobulinemia these must be evolved from the same cells (27 38)

As a rule, it is stipulated that in definite cases of primary macroglobulin emia there should be a sedimentation constant higher than 16 S (11) although a few authors accept a lower value (12—13 S) (15 27 29)

The concentration of serum proteins influences the sedimentation properties (17) and for this reason it is sometimes difficult to compare the numerous earlier publications. For the same reason the S-value is not always useful in the differen tial diagnosis between primary macroglobulinemia and multiple myeloma (26)

In our case, however an attempt has been made to surmount this difficulty by repeating the ultracentrifugation with smaller protein concentrations, where the conditions are more ideal This showed an increase in the sedimentation coeffi cient such that the sedimentation constants extrapolated to zero concentration are $s = 18$ S and $s = 19$ S for macroglobulins. The overall evaluation of the ultracentrifugal tests thus suggests a primary macroglobulinemia, even though it is unusual to find a division of the macroglobulin apex as seen here.

With free serum electrophoresis or paper-electrophoresis M-components appear as a high narrow peak in the globulin fraction. Macroglobulins and myeloma proteins cannot be separated by this test (31) A few sera have with paper electrophoresis apparently contained 2 M-components, but recent investigations have shown that the phenomenon comes from aggregation between an M-component and other proteins, mainly albumin (11)

Immunoelectrophoresis is far better than the conventional electrophoresis to demonstrate the pathological changes in myelomatosis and primary macroglobulinemia. In multiple myeloma 3 types can be distinguished γ-myeloma β_2A myeloma and a micro-molecular type ($\gamma\mu$) In primary macroglobulinemia a distinct spread β M curve (5 11) is found Increased amounts of the above globulins are, as a rule, accompanied by a reduction in the amount of the other immune globulins. Lately there has, how ever been observed in two patients a simultaneous increase in 2 myeloma proteins (10) which is just as unusual as the simultaneous appearance of a β M macroglobulin and a γ-myeloma protein.

The case described hardly appears to fall under the heading of either primary macroglobulinemia or multiple myeloma. Possibly it has been one of the unusual transitional forms between the two diseases but the diagnosis must remain uncertain until there appear better criteria for distinguishing between these two diseases.

Summary

An unusual case of "paraproteinemia" is described, where the patient's serum at the same time contained 2 M-components, a β_2-M macroglobulin and a γ-myeloma protein. 36 % of total protein consisted of components with the sedimentation constants s = 18 S and s = 19 S (extrapolated to zero concentration) there was, however 1.5 % of this amount, which had s = 31 S (normal dilution) Sternal marrow showed a slight lymphocytosis. Possibly the case represents one of the rare transitional forms between myelomatous and primary macroglobulinemia. — A short literature survey is given.

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Wissler's Syndrome[1]

By

L. E. Böttiger and J. Landgren

In 1944 Wissler (10) described a syndrome now appearing under the name of subsepsis allergica Wissler. The main characteristics are: high intermittent fever, irregular recurring exanthemata of different types, neutrophil leukocytosis, increased sedimentation rate, negative cultures, no demonstrable cause and good prognosis. The syndrome has been described in a score of cases, exclusively in the paediatric literature, that was reviewed by Vestermark in 1960 (9).

We have had the opportunity to observe two cases above 15 years of age, presenting most of the characteristics mentioned and presumably belonging to this disease entity. Both were treated at departments of internal medicine. Although the syndrome is rare, it seems to be diagnosed with an increasing frequency and as the diagnosis is difficult to reach, we think the knowledge of the condition should be made known even to internists. In addition, some new aspects on the pathogenesis will be presented.

Submitted for publication March 23, 1963.

Case reports

Case 1 (previously reported by Böttiger (3)). A 16-year-old boy was admitted for the first time in 1952 because of fever and arthralgia. He had previously been healthy. For two months before admission he had been suffering from a condition that started as an upper respiratory disease with fever and continued as bouts of high fever even after the respiratory symptoms had disappeared.

Physical examination on admission revealed a normally developed boy with slight pharyngitis and some enlarged nodes at the mandibular angle. He complained of pain in the joints but showed no signs of arthritis. A soft apical systolic murmur was heard. Laboratory findings were normal on admission, with the exception of an elevated ESR (31 mm/hr — Westergren method). Bacterial cultures were negative or showed ordinary findings (a throat culture showed growth of *Staphylococcus albus*). Two weeks later he developed *increasing migratory joint pains*, now with some swelling and tenderness of a few distal interphalangeal joints and of the right wrist, a maculopapular rash and fever spiking up to 40.6° C. Hb 13.3 g %, ESR 84 mm/hr and WBC 14 700 with a moderate

[1] This article is number VIII in a series of clinical studies on fever of unknown origin.

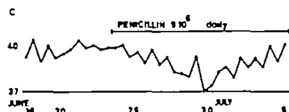


Fig 1 Part of temperature chart of case 1 showing initial response to penicillin treatment.

shift to the left. Repeated blood cultures were negative as were febrile agglutinins. No LE-cells were found. He was treated with various antibiotics as well as with cortisone in a dosage of 100 mg daily without effect. The fever slowly subsided and he was discharged with a questionable diagnosis of systemic lupus erythematosus. He was later admitted in 1953 1954 1955 and 1956 on all occasions with an identical clinical picture. (ESR varying from 15—118 mm/hr Hb 9.2—12 7 g % with a low serum iron, WBC 4,300—27 600 differential count with a shift to the left and normal number of eosinophils.) On one occasion transient electrocardiographic signs of myocardial involvement (T wave changes) were found but the patient had no cardiac symptoms or signs of cardiac insufficiency On the third admission (1954) the earlier demonstrated staphylococci (positive throat cultures and one blood culture with a sparse growth of Staphylococcus albus) together with the rising antistaphylolysin titer (4 4—5 6 —16 0 units/ml) made us suspect staphylococci as the etiological agent. At that time erythromycin had become available and he was treated with that drug with a dramatic response. The fever subsided rapidly the leukocytosis disappeared and the ESR dropped to normal values. When he was re-admitted in 1955 with the same clinical picture the antistaphylolysin titer was again rising but erythromycin now was ineffective (resistant bacteria?) Penicillin in large doses (9 million units daily) lowered the fever initially (fig 1) which however again rose and continued for several weeks. After a last short admission in 1956 he was put on an antihistamine (chlorcyclizine) which obviously was successful in preventing or cutting short further attacks of arthralgia and rash. He has been healthy ever since and a control in January 1963 showed him to be in excellent health, capable of heavy work as a farmer

Case 2 A 16-year-old school girl had had measles, mumps and otitis as a small child, but had otherwise been healthy She had had two attacks of upper respiratory disease with fever four and two months before admission.

She was admitted to the hospital in August 1962 after she had fallen acutely ill with fever and developed abdominal pain. An appendectomy was performed but there were no abnormal findings in the abdomen and the appendix was normal, even on microscopical examination. She was transferred to the department of medicine as a case of pyrexia of unknown origin

Her temperature chart is reproduced in fig 2 On admission she was in no acute distress and physical examination revealed a pale but otherwise healthy looking young girl without any abnormal findings except the fever A soft systolic murmur was heard over the apical region of the heart. Laboratory examinations Hb 11.8 g % WBC 8,500 with a normal differential count. Platelets 108,000, ESR 55 mm/hr (Westergren) Urinalysis negative. Febrile agglutinins negative, tests for toxoplasmosis, infectious mononucleosis and RSSE negative. Repeated cultures from sputum, urine, cerebrospinal fluid arterial and venous blood did not reveal anything abnormal Repeated examinations for LE cells were all negative. Serum bilirubin 0.2 mg S-GOT and S-GPT initially were within normal limits. After one month they were checked routinely and found to be markedly elevated (300 and 500 units respectively) without any clinical symptoms of hepatic disease. Needle biopsy of the liver on this occasion showed a liver parenchyma with some devitalized cells but nothing definitely abnormal (normal liver architecture and no inflammatory reaction) *Her serum enzymes* rapidly returned to normal and she has developed no signs of hepatic failure or portal vein obstruction. Serum electrophoresis showed low albumin, elevated α_2 and normal γ-globulins. Haptoglobins 500 mg % Stool guaiacs negative Protein-bound serum iodine 7 05 γ % Sternal marrow was normal on two occasions. Cerebrospinal fluid normal. Eye grounds normal. Extensive X-ray examinations (gastrointestinal tract, intravenous

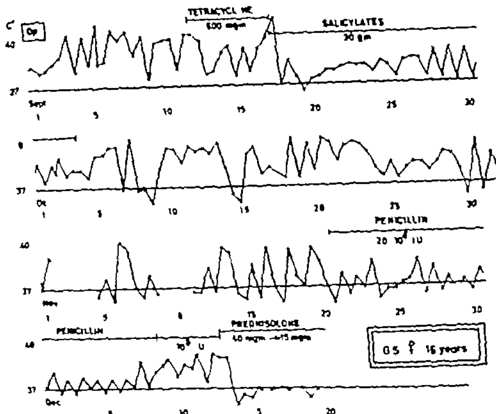


Fig. 2. Complete temperature chart of case 2. Note initial response to penicillin treatment (end of November — beginning of December)

pyelograms, lungs, nasal sinus, teeth and skeleton) without any abnormal findings.

Although she had chills when the fever rose, she was always remarkedly unaffected by the disease and had no other complaints, her general condition was always good. There were never any signs of cardiac involvement, and the innocent systolic murmur did not change in character. A therapeutic trial (see fig. 2.) with tetracyclines (600 mg daily) was without effect. Salicylates (3 g daily) had some initial effect on the fever but the effect could not be maintained. Antistreptolysin titers were normal, antistaphylolysin titers were slowly rising (1.0—2.0—2.8 units/ml) until penicillin was given, when again they went down to 1.4 units/ml. Her ESR varied between 31 and 100 mm/hr the serum iron was low the hemoglobin content between 7.3 and 12.2 g % and the WBC between 4,100 and 15 400 with increased amount of young forms and normal percentage of eosinophils.

Finally after three months in the hospital prednisolone was started (initial dose 40 mg/day) with a dramatic response. Her temperature became immediately normal and she was discharged a week later. She was on a small dose of prednisolone (1.25 mg × 2) but this dose was insufficient to keep the temperature entirely normal until an antihistamine was added (chlorcyclizine). She is well and without prednisolone eight months after discharge.

Discussion

These two patients showed almost identical pictures. They had *an irregular fever* with high peaks and chills they had

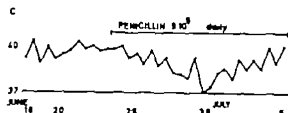


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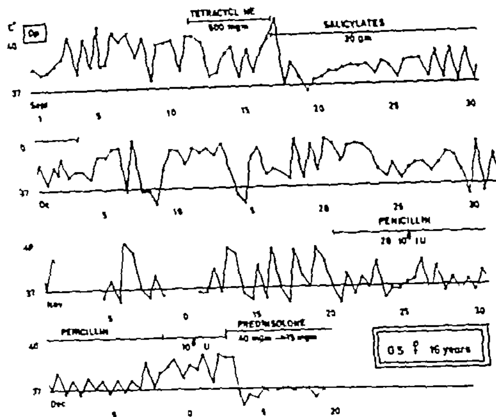


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Finally after three months in the hospital prednisolone was started (initial dose 40 mg/day) with a dramatic response. Her temperature became immediately normal and she was discharged a week later. She was on a small dose of prednisolone (1.25 mg × 2) but this dose was insufficient to keep the temperature entirely normal until an antihistamine was added (chlorcyclizine). She is well and without prednisolone eight months after discharge.

Discussion

These two patients showed almost identical pictures. They had an *irregular fever* with high peaks and chills, they had

a leukocytosis of 15—25 000 with a moderate shift to the left and normal number of eosinophils they had a marked elevation of the ESR (maximum values 85 and 118 mm/hr respectively) and anemia. Both always appeared *unaffected by the fever* and no cause for their symptoms could be found One had varying cutaneous manifestations and transient electrocardiographic signs of myocardial damage, the other on one occasion had signs of hepatic involvement. It should be noted that in addition to fever leukocytosis and an elevated ESR reports have been made of "nephropathy (2 4 5 7) "hepatitis" (2 5 6) "pericarditis (4 8) "pleuritis (2) and a moderately enlarged spleen (7)

Both cases fit well into Wissler's description of what he named subsepsis hyperergica and Fanconi (3) later renamed subsepsis allergica Wissler The literature of the Wissler syndrome has been reviewed by Vestermark (9) where references may be found to the previously published cases. It seems probable that the patients with this kind of fever are more numerous than may be concluded from the published reports and Zetterström (11) has stated that paediatricians aware of the syndrome see an increasing number of cases. The two adolescents described here are older than any of the patients reported upon previously and make it essential that not only paediatricians but also internists should be familiar with the syndrome. It is quite likely that it represents a juvenile way of reacting and that less dramatic pictures should be watched for in the adult patients. Perhaps the Wissler syndrome is the explanation of some of the cases with fever of unknown origin and good prognosis reported in studies of fever of unknown origin.

Both the patients had a rise in antistaphylolysin titer The boy had a very marked increase with a maximum value of 22 units/ml and a decrease after erythromycin treatment. The girl had a more moderate increase, but in a comparatively early stage of her disease she was treated with massive doses of penicillin which may have stopped a further increase and actually seemed to effect a decrease of the titer Both patients showed an initial response to massive doses of penicillin (figs. 1 and 2) This could imply that *staphylococci were involved* in the production of the syndrome, which would also be supported by the fact that staphylococci were found on numerous occasions in throat cultures in the first case We have not been able to find notes of the antistaphylolysin titer in more than one previously recorded patient (9) in whom it was found to be elevated. One would have to postulate that there exist individuals with a special reactivity of their connective tissues and that these individuals do react to staphylococci in a way very similar to the one in which other individuals react against streptococci with scarlet fever and rheumatic fever Fanconi actually said that "*cum grano salis* scarlet fever could be looked upon as a special type of subsepsis Both our patients as many of the previously published ones, had repeated slight upper respiratory infections before the outbreak of the Wissler syndrome. The repeated attacks would fit well with a theory of sensitization against staphylococci and make it likely that the responsible bacteria resided in the pharynx

The diagnosis is difficult and can only be reached by exclusion of other known fever producing diseases. The main entities that enter into the differential diag

nosis are collagen diseases and various infections. Some of the patients previously reported have later developed rheumatoid arthritis, which might point to a connection between the Wissler syndrome and the collagen diseases. The clinical picture in many aspects is very similar to rheumatic fever and systemic l pus erythematosus and would suggest a reaction in the connective tissues that would place th Wissler syndrome in the group of collagen disease". The Wissler cases might also be interpreted as cases of collagen disease with a superimposed staphylococcal infection. However again, the good prognosis and the normal level of plasma gammaglobulins place the Wissler patients in a different group.

Although an infection may start the reaction, t seems definite that the essential character of the disease is not infectious, and this implies that the name subsepsis should be abandoned. Other names have been suggested. We would prefer to call the disease the Wissler syndrome until more is known about the etiology.

Various therapeutic agents have been tried (cf 9). It is essential to exclude the possibility of active infection. Steroids may then be tried in moderate or large doses. The possibility that staphylococci may be of etiological significance should be taken into consideration, the antistaphylolysin titer should be followed and massive therapy against staphylococci instituted if a staphylococcal infection is found or strongly suspected.

Antihistamines in our opinion have been helpful to alleviate the symptoms.

Summary

Two cases of Wissler' syndrome are described. The etiology is discussed and it is suggested that staphylococci may be involved in the pathogenesis of the syndrome.

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Addendum

After the submission of the manuscript another identical case has been brought to our notice through the courtesy of Drs. W. T. L. Ohlsson and B. Hyang, Ängelholm.

A 16-year-old girl gets an upper respiratory infection and one week later migratory pains in muscles and joints. On admission to the hospital physical examination reveals slight redness of the pharynx and a

friction rub over the precordial area, otherwise normal conditions. Laboratory findings ESR 105 mm/hr Hb 10 9 % WBC 20.100 Repeated cultures negative, serological examinations negative, except *the antistaphylolysin titre that rises from 2.5 on admission to 6.2 units/ml* 8 weeks later She also gets hepatic involvement with increased S-GOT and S-GPT values, but normal BSP-retention. Four months after she fell ill she is in good condition without signs of remaining damage to heart or liver but still on a small dose of prednisone and antihistamin.

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Serum β-Lipoprotein Lipids and Protein in Elderly Male Survivors of Myocardial Infarction

By

Kim Cramér

The work on serum lipoproteins in myocardial infarction has been focused mainly on the lipid content of the lipoprotein or on the lipoprotein concentration in serum. Smith (13) recently gave figures on the composition of the Sf 0—1 subclass of the β-lipoproteins, and found that it was constant regardless of the serum concentration of this class. It is unlikely however that any substantial abnormalities could be demonstrated in lipoproteins isolated by ultracentrifugation, where a more or less constant lipid/protein relationship is essential for the isolation. When isolation by chromatography on hydroxylapatite was performed (10) it was possible to demonstrate increasing cholesterol/protein ratios in the β-lipoproteins with increasing age in both sexes, and higher cholesterol/protein ratios in elderly males than in elderly females (5). The phospholipid/protein ratios, on the other hand, were found to be constant within all age groups. The isolation of β-lipoproteins on hydroxylapatite also makes possible a determination of the serum concentration of lipoprotein protein, which is difficult to achieve through ultracentrifugation.

The aim of this investigation was to find out whether the high β-lipoprotein cholesterol/protein ratio in normal elderly males could also be found in survivors of myocardial infarction in the same age group, and if any of the β-lipoprotein components could be found to be increased in concentration.

Clinical material

All patients admitted to Medical Service I of Sahlgrenska sjukhuset during the period April 1st 1960—March 31st 1961 were studied with serum lipid determination during the acute course of the disease and 3 and 12 months following the onset of the infarction. Details of this study are given in a separate publication (17). The patients were kept on a fat-restricted diet during their hospital confinement, but did not adhere to this after discharge. One year after the infarction, none of them observed any special dietary restrictions. Of the total number of 22, in whom complete series of observations were made, 12 males

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Table I Concentration of serum lipids and of β-lipoprotein components in a normal material (5) and in survivors of myocardial infarction. Both materials consist of males 55—65 years of age. P values are given where significant differences were found

	Serum			β-lipoprotein			
	Cholesterol (mg/100 ml)	P lipid (mg/100 ml)	Glycerides (mMol/l)	Cholesterol (mg/100 ml)	P-lipid (mg/100 ml)	Glycerides (mMol/l)	Protein N (mMol/l)
11 normal males (55—65 years)	213 ± 9	236 ± 10	0 79 0.70–0.90	173 ± 10	125 ± 7	0.56 0.49–0.63	13.89 ± 0.83
12 male survivors of myocardial infarction (55—65 years)	253 ± 12 < 0.02	247 ± 13	0.94 0.81–1 08	185 ± 12	146 ± 9	0.66 0.56–0 78	17 19 ± 0.91 < 0.02

Table II Ratios between β lipoprotein lipids and β lipoprotein protein nitrogen and ratios cholesterol/phospholipids in the β-lipoproteins in the same materials as in table I. P values are given where significant differences were found

	β-lipoprotein composition in molar ratios				
	Lipid/10 moles protein nitrogen			Cholesterol/P-lipid	
	Cholesterol	P lipid	Glycerides	Molar	Weight
11 normal males (55—65 years)	3.24 ± 0.06	1 14 ± 0 03	0 42 ± 0.05	2.80 ± 0.07	1 40 ± 0.04
12 male survivors of myocardial infarction (55—65 years)	2.78 ± 0.08 < 0.001	1 09 ± 0.02	0.44 ± 0.07	2.55 ± 0.05	1.28 ± 0.03 < 0.01

were within the age group 55—65 years and are included in this study. Their sera were all examined between 13 and 18 months after the acute onset of the infarction. None of them had had any sign of reinfarction in the meantime. The values are compared with values from 11 clinically healthy males, who had no family history of atherosclerotic disease (5)

Methods

All blood specimens were withdrawn after a 12 hour fast in the morning. The β-lipoproteins were separated by chromatography on hydroxylapatite (10) using buffer solutions with 0.2 % EDTA (for further details see (5))

Lipid determinations were performed on a 1 1 chloroform/methanol (v/v) extract (15) Chlolesterol was determined as described by Cramér and Isaksson (9) phospholipids according to Svanborg and Svennerholm (16) glyceride glycerol according to Carlson (3) The cholesterol values were reduced by 4.6 % (1 9) in order to make them comparable with values obtained by the method of Sperry and Webb (14)

The distribution of glyceride values was skew and the values were transformed into logarithms before statistical calculations.

Results

The serum cholesterol values were higher in the infarction group, while the two groups showed no differences in the values for serum phospholipids or glycerides (table I) β-lipoprotein lipids were found to be at the same level in both groups, while there was an increase in β-lipoprotein protein in the infarction group ($p < 0.02$)

The molar ratios between the β-lipoprotein components showed lower cholesterol/protein ratios in the infarction group ($p < 0.001$) as well as lower cholesterol/phospholipid ratios (table II) ($p < 0.01$)

The cholesterol and phospholipid moieties of the β-lipoprotein were both significantly correlated to the protein ($r = 0.92$ for cholesterol, 0.95 for phospholipids, $p < 0.001$) while a somewhat lower correlation was found for glycerides and protein ($r = 0.68$, $p < 0.01$)

Discussion

The serum lipids in this group of elderly males showed an elevation only of cholesterol which greed with a corresponding age group investigated by Carlson (4) Carlson also found an elevation of β-lipoprotein cholesterol which was not present here. The general elevation of the β-lipoprotein lipid, which has been demonstrated by numerous workers in myocardial infarction in younger age groups, does not appear to be compulsory in the elderly patients.

Data on the β-lipoprotein composition, including determinations of lipoprotein protein, have been published on a similar material only by Smith (13) Her values for the Sf 0—12 class, which was prepared in the ultracentrifuge, show a greater abundance of cholesterol than has been reported by any other investigator The cholesterol/phospholipid ratio in her material averages 2.02 (molar ratio = 4 04) while the highest previously reported figure for the same fraction, found by Oncley et al. (12) is 1 44 (molar ratio 2.88)

The preparations from hydroxylapatite columns are immunologically homogeneous and contain lipoproteins of the density classes from 1.063 down to below 1.006 (8) The antigenic characters of the lipoprotein protein are similar within the density range of 1 063—0.96 (2, 11) The values for lipoprotein protein, which are given here, thus represent the lipid-carrying vehicle within a wide range of lipoprotein densities.

As could be expected, they are considerably higher than Smith's values for a more limited density class, which can be calculated to 10.82 mMol protein nitrogen/l for the ischemic heart disease group and to 5.33 mMol/l for the healthy control group

A conversion of the figures obtained (assuming a 3 1 ratio for esterified/free cholesterol as found by Smith) to percentage weight composition gives the following figures for comparison with Smith's material of patients with ischemic heart disease

	Protein	Cholesterol Ester	Cholesterol Free	Glycerides	Phospholipids
Present material	23.3	37 1	7.3	10.0	22.4
Smith (13)	16.9	48.0	9.3	8.6	17 1

A common feature of both materials is the constant proportions between protein and phospholipids. It has been demonstrated that this ratio is very constant also during rapid shifts in β-lipoprotein serum concentration (5, 6 7 13)

Summary

The concentration of β-lipoprotein components and the composition of the β lipoproteins were examined in 12 male survivors of myocardial infarction in the age group 55—65 years. Isolation of β-lipoprotein by chromatography on hydroxylapatite was applied Values from 11 healthy men of the same age were used as controls. An elevation of β lipoprotein protein ($p < 0.02$) was found in the infarction group but there was no elevation of the β-lipoprotein lipids The cholesterol protein ratio was lower ($p < 0.001$) as was the cholesterol/phospholipid ratio ($p < 0.01$)

Acknowledgment

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Comparative Studies on the Diuretic Effect of Chlorothiazide and Spironolactone in Cardiac and Hepatogenic Oedema

By

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It has been amply substantiated that a synergistic effect is exerted by diuretics of the chlorothiazide type and the aldosterone antagonists of the spironolactone group (10, 15, 23, 24, 26, 28). Frequently a good diuretic effect of this combination has been obtained even in cases where each agent separately has been ineffective.

The theoretical basis of this synergism has been elucidated by several studies (15, 21, 24, 28). It is explained by the different sites of action of the agents in the proximal and distal tubules of the kidney. And an ideal diuretic treatment should affect both. If not, inhibition of re-absorption in one site may be rendered ineffective by increased re-absorption in the other site.

In the pathogenesis of oedema the mechanism responsible for the renal retention of sodium, including the possible role of secondary hyperaldosteronism or increased renal sensitivity to aldosterone, is still far from clear. In this connection it would seem of some interest to learn whether in oedema of pathogenesis so (presumably) different as cardiac and cirrhotic there is the same response to diuretics so different in their renal sites of action as chlorothiazide and the aldosterone antagonists. In an attempt to throw some light on this question, we set out to compare the effects of these two types of diuretic administered separately to a series of patients of the named categories.

In some of the cases, moreover, we compared the effect of these diuretics with the diuretic effect of prednisone which has been reported in a number of oedematous conditions, cardiac (11, 25) as well as cirrhotic (4, 10, 27, 32). The diuretic effect of prednisone has been explained partly as an increase in "free water clearance" (27, 32) giving an increased water diuresis, partly as an inhibition of an increased production of aldosterone (4, 11, 2) which will, furthermore, result in natriuresis. This inhibi-

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Summary

The concentration of β lipoprotein components and the composition of the β-lipoproteins were examined in 12 male survivors of myocardial infarction in the age group 55—65 years Isolation of β lipoprotein by chromatography on hydroxylapatite was applied Values from 11 healthy men of the same age were used as controls An elevation of β-lipoprotein protein ($p < 0.02$) was found in the infarction group but there was no elevation of the β-lipoprotein lipids. The cholesterol protein ratio was lower ($p < 0.001$) as was the cholesterol/phospholipid ratio ($p < 0.01$)

Acknowledgment

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Table II. Effects of chlorothiazide and spironolactone in severely decompensated heart disease

Case no. Sex	Diet (mEq Na) (about)	Increase in sodium output (mEq N)				Exch. sodium		Blood volume		Haemato-crit (%)	Body weight (kg)
		Chloro-thiazide	Spirono-lactone	Chloro-thiazide + spirono-lactone	Prednisone	mEq Na	mEq/kg	l	ml/kg		
1 ♀	35	—	—	—	—	4,700	52	—	—	—	90
		—	800	—	—	3,710	46	—	—	—	81
		100	—	—	—	3,400	43	—	—	—	80
			—	—	—	4,080	53	—	—	—	85
		900	—	—	—	4,200	53	—	—	—	80
		—	1 000	—	—	3,580	47	—	—	—	77
		—	—	—	400	—	—	—	—	—	76
2 ♀	85	—	—	—	—	4 310	96	—	—	—	45
		—	1,400	—	—	3,930	102	—	—	—	39
		400	—	—	—	2,590	72	—	—	—	36
		—	—	—	500	—	—	—	—	—	34
3 ♀	35	—	—		—	5,400	83	—	—	—	65
		1,500	—		—	4,140	77	—	—	—	54
		—	400		—	3 400	71	—	—	—	48
4 ♂	83	—	—		—	7,840	97	—	—	—	81
		—	100	—	—	—	—	9.60	124	47	77
		—	—	700	—	6,420	87	9.35	129	46	74
		100	—	—	—	7 610	94	9.70	131	50	74
		—	—	—	—	7,240	88	9.60	130	51	74
5 ♂	85	—	—	—	—	—	—	6.08	74	45	82
		100	—	—	—	—	—	6.23	74	43	84
		—	100	—	—	7,580	84	6.30	72	44	86
		—	—	—	0	—	—	—	—	—	—
6 ♀	83	—	—	600	—	8,600	83	6.03	75	43	80
		—	50	—	—	4,250	61	7.28	104	46	70
		—	—	500	—	—	—	—	—	—	—
		50	—	—	—	3,650	54	6.63	99	43	67
7 ♂	83	—	—	—	—	—	—	—	—	—	—
		100	—	—	—	6,150	72	8.12	97	46	88
		—	2,000	—	—	6,550	74	—	—	—	—
			Respirator			3,190	—	5.78	—	47	—

Experimental data shown also in figs. 3 and 4.

Methods

Chlorothiazide was administered in a dosage of 500 mg twice daily for 3 days, spironolactone[1] in a dosage of 300 mg three times daily for 6 days, and prednisone in a dosage of 10 mg three times daily for 5 days. Spironolactone and prednisone were administered

[1] The aldosterone antagonist spironolactone was administered in its original, non-micronized form as Aldactone (Searle) 1 tablet = 100 mg.

Table I Effects of chlorothiazide and spironolactone in normal subjects

Case no. Sex	Diet (mEq Na) (about)	Increase in sodium output (mEq Na)			Exch. sodium		Blood volume		Haematocrit (%)	Body weight (kg)
		Chlorothiazide	Spironolactone	Prednisone	mEq Na	mEq/kg	l	ml/kg		
1 ♂	35	—	—	—	—	—	—	—	—	75
		200	—	—	—	—	—	—	—	73
		—	50	—	—	—	—	—	—	73
2 o	35	—	—	—	—	—	—	—	—	54
		—	150	—	—	—	—	—	—	52
		250	—	—	—	—	—	—	—	52
3 ♂	35	—	—	—	—	—	—	—	—	65
		—	300	—	—	—	—	—	—	64
		150	—	—	—	—	—	—	—	64
4 ♀	85	—	—	—	2,360	43	3.70	67	35	55
		250	—	—	—	—	3.75	69	35	54
		—	150	—	1,920	36	3.50	66	36	53
		—	—	100	2,700	50	3.50	65	37	54
5 ♀	85	—	—	—	—	—	3.90	61	38	64
		150	—	—	3 000	48	3.40	55	42	62
		—	200	—	2,900	48	3.20	53	44	60
		—	—	0	—	—	—	—	—	60
6 ♀	85	—	—	—	2,600	45	3.70	62	39	60
		—	300	—	2,200	37	3.10	53	40	59
		50	—	—	—	—	3.15	53	38	59
		—	—	50	—	—	—	—	—	59

Experimental data shown also in figs. 1 and 2.

tion might be imagined as operating via an inhibited pituitary function. Although aldosterone production is largely independent of pituitary function — hypophysectomized dogs still responding to blood loss by an increased aldosterone secretion — this response and moreover the basal secretion have been found to be considerably reduced following hypophysectomy (7) *In vitro* experiments have shown a stimulatory effect of ACTH upon the synthesis of aldosterone in sections from adrenal tissue (13) and Addisonian patients can increase their aldosterone production when stimulated by ACTH (20)

Material

A. Fourteen patients with cardiac failure due to arteriosclerotic heart disease (except case 7 in table II and case 4 in table III who had cor pulmonale and mitral disease respectively) including 7 with severe decompensation severe oedema and severe pulmonary as well as hepatic congestion 4 with moderate decompensation pulmonary congestion, a moderate tendency to oedema and palpable hepatomegaly and lastly 3 with mild decompensation pulmonary congestion, but no signs of hepatic congestion and no peripheral oedema

B. Six patients with chronic parenchymal liver disease, including 3 with oedema and ascites.

C. Six subjects without oedema, without hypertension, and without signs of cardiac, hepatic, or renal disease.

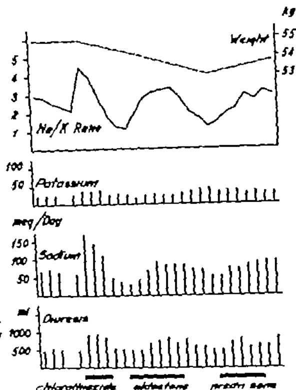


Fig. 2. Effects of chlorothiazide, spironolactone and prednisone upon the urinary output of sodium and potassium in normal subject (table I case 4)

aldosteronism may entail an equivalent urinary excretion of potassium, 10 patients received, during the pre-treatment period, 85 mEq sodium in the form of NaCl tablets. Neither normal subjects nor patients with cardiac or cirrhotic oedema showed any directly demonstrable increase in the excretion of sodium or potassium. This test was omitted in the remaining cases.

Table II presents the results in the 7 patients with severe cardiac decompensation. Three were also treated with prednisone. Case 1 was studied in two periods in which the initial values of exchangeabl sodium were approximately the same. In the former period spironolactone gave a good effect and chlorothiazide a questionable effect. In the latter period a good effect was found both with chlorothiazide and with spironolactone and some effect with prednisone. Fig 3 illustrates the results in case 2 of table II and shows a considerable effect of spironolactone and some effect of pred nisone and chlorothiazide upon the sodium output.

In case 7 (fig. 4) of this table the treat ment schedule had to be changed. This patient had a chronic pulmonary disease with cor pulmonale, and during the stay in hospital he showed respiratory failure such that he required tracheot omy and espirator treatment. It will be seen that neither chlorothiazide nor mercaptomerin (Thiomerin, a mercurial diuretic) had any diuretic effect during the state of respiratory failure, while

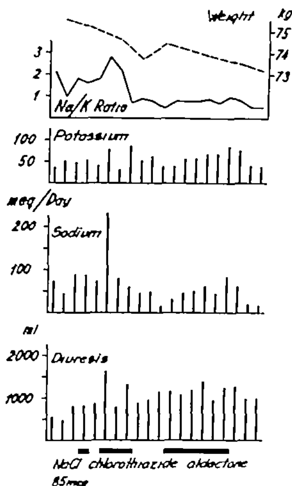


Fig 1 Effects of chlorothiazide and spironolactone upon the urinary output of sodium and potassium in a normal subject (table I case 1)

longer than chlorothiazide owing to their presumed longer latent period before an effect may be obtained.

Patients admitted on odd dates were treated first with chlorothiazide and then with spironolactone, while those admitted on even dates received spironolactone first In 13 cases prednisone was administered in a third treatment period Lastly 3 patients had a combination of chlorothiazide and spironolactone in the above doses for 6 days. The intervals between the treatment periods were 3—5 days.

During the period of the study no changes were made in digitalis medication, if any and no other drugs known to exert a diuretic effect were administered. No patient had a supplement of potassium. The dietary content of sodium was in some cases about 35 and in others about 85 mEq/24 hours.

The patients were followed by daily measurements of the urinary output and determination of the 24-hour output of sodium and potassium (Eppendorff flame photometer) On this basis, the increase in the sodium excretion during the treatment periods was roughly estimated. Complete balance studies were not performed

In 13 and 22 cases respectively repeated determinations of blood volume and exchangeable sodium were carried out. The blood volume was determined by ^{131}I-labelled albumin by means of a volumetron apparatus (Atomium, Perkin Elmer) as advocated by Williams & Fine (31) The dosage was about 3 μC per determination. The standard deviation in connection with the countings was 2—4 %

Exchangeable sodium was determined by the long-lived sodium isotope ^{22}Na using an equilibration period of 20—24 hours (17 18). The sodium pool thus determined corresponds in normal subjects to about 70—80 % of the total sodium content. Correction was made for the urinary excretion of sodium and for a simultaneous ^{131}I activity in the serum and urine. Owing to the relatively long biological half life of ^{22}Na we used a dosage of only 3μC per determination. The measurement of the serum activity was carried out in a well type scintillation crystal detector with a pulse height analyser (Tracerlab) Using this method the standard deviation at the first determination is 3 % increasing in later determinations to 6 %

Results

Table I gives the results in the 6 normal subjects. Figs. 1 and 2 show the detailed results in cases 1 and 4 (table I) The increase in the excretion of sodium is seen to be small (< 300 mEq) in all cases, although in several treatment periods it was unmistakable (figs. 1 and 2) and evident particularly from the urinary Na.K ratio

Inspired by Hadorn s (12) assumption that a moderate sodium intake in hyper

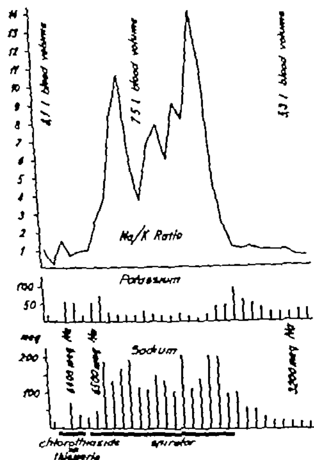


Fig. 4. Effects of chlorothiazide and spironolactone treatment upon the urinary output of sodium and potassium in patient with severely decompensated heart disease (table II, case 7)

Table III sets out the results in 4 patients with moderate (cases 8—11) and 3 with mild (cases 12—14) cardiac decompensation. In this series 2 were treated with prednisone. The determinations in case 11 are shown in fig 5. Among the moderately decompensated patients chlorothiazide as well as spironolactone exerted an acceptable effect in 1 out of 4 (case 8 with chlorothiazide and case 11 with spironolactone). Prednisone had moderate effect in one out of one (case 11). Among the patients with mild decompensation the corresponding values for chlorothiazide and spironolactone were 1 out of 3 (case 14 with chlorothiazide and case 13 with spironolactone) and for prednisone 0 out of 1.

Within the entire group of cardiacs there was, then, an effect of chlorothiazide in 5 out of 14, of spironolactone in 5 out of 13, and of prednisone in 3 out of 5. Two who responded to chlorothiazide failed to respond to spironolactone. Reversely 2 who responded to spironolactone alone proved to be resistant to chlorothiazide. In the 3 cases in which prednisone had a diuretic effect, spironolactone also had an effect, each time considerably greater. Otherwise there was

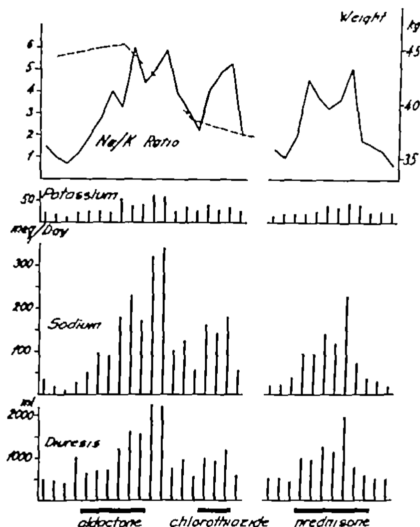


Fig 3 Effects of spironolactone chlorothiazide and prednisone upon the urinary output of sodium and potassium in a patient with severely decompensated heart disease (table II case 2).

respirator treatment was rapidly followed by a high sodium output accompanied by a fall in potassium output resulting in by far the highest urinary Na.K ratio found in the entire series. It is worth noting that the time at which the sodium output decreased again and the potassium output increased coincided remarkably with the time at which he could manage on spontaneous respiration. During the respirator treatment the value of his exchangeable sodium fell to one half and the blood volume to two-thirds of the initial values. It may be mentioned that during these violent electrolyte and fluid changes the sodium and potassium concentrations in the serum as well as the haematocrit value remained almost unchanged and within the normal range

Table II shows, moreover that in these patients with severely decompensated heart disease there was a moderate to good effect of chlorothiazide in 3 out of 7 (cases 1 2 and 3) (> 300 mEq Na) of spironolactone in 3 out of 6 (cases 1 2, and 3) and of prednisone in 2 out of 3 (cases 1 and 2) The combination of chlorothiazide and spironolactone had a favourable effect in 3 out of 3 in whom each agent separately had proved ineffective (cases 4 5 and 6)

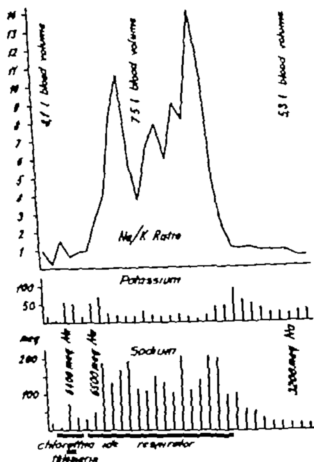


Fig. 4. Effects of chlorothiazide and respirator treatment upon the urinary output of sodium and potassium in patient with severely decompensated heart disease (table II, case 7

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Table III Effects of chlorothiazide and spironolactone in moderately decompensated heart disease (cases 8—11) and heart disease with mild decompensation (cases 12—14)

Case no. Sex	Diet (mEq Na) (about)	Increase in sodium output (mEq Na)			Exch. sodium		Blood volume		Haematocrit (%)	Body weight (kg)
		Chlorothiazide	Spironolactone	Prednisone	mEq Na	mEq/kg	l	ml/kg		
8 ♂	35	—	—	—	4 880	72	—	—	—	68
		—	150	—	4 440	64	—	—	—	67
		500	—	—	3,863	66	—	—	—	59
		—	—	—	5,110	77	6.30	96	34	66
9 ♂	35	—	—	—	—	—	—	—	—	70
		—	150	—	—	—	—	—	—	67
		300	—	—	—	—	—	—	—	63
10 ♂	35	—	—	—	3,530	57	—	—	—	62
		150	—	—	2,900	49	—	—	—	59
		—	50	—	3,160	54	—	—	—	59
11 ♀	85	—	—	—	—	—	—	—	—	57
		—	450	—	—	—	—	—	—	56
		200	—	—	3,290	59	—	—	—	—
		—	—	400	3,120	55	—	—	—	57
12 ♀	35	—	—	—	2,500	43	—	—	—	58
		150	—	—	2,520	44	—	—	—	57
		—	150	—	2,660	48	—	—	—	56
13 ♂	85	—	—	—	4,170	47	—	—	—	88
		250	—	—	3,670	42	—	—	—	87
		—	500	—	—	—	—	—	—	86
		—	—	0	3,900	44	—	—	—	88
14 ♂	85	—	—	—	3,340	52	4 16	67	—	62
		400	—	—	—	—	4.35	73	—	60
		—	200	—	2,670	45	4.78	80	42	60

Experimental data shown also in fig 5.

no striking correlation between the diuretic effects of the three agents.

Table IV shows the results in 6 patients with hepatic cirrhosis, 3 (cases 1—3) without and 3 (cases 4—6) with oedema and ascites. Figs 6 and 7 give the results in one patient of each category In 5 the effect of prednisone was studied. There was an effect of chlorothiazide in 3 out of 6 (table IV cases 2 3 and 6) while an effect of spironolactone was found in 6 out of 6 A slight effect of prednisone was found in only one patient (case 2)

One patient developed a rapidly transient generalized exanthema towards the end of the spironolactone treatment. Apart from this, there were no reactions which could be interpreted as side effects of the drugs, in particular no patient had dyspeptic complaints on spironolactone (28)

As far as the diet is concerned, the patients of the series did not receive the

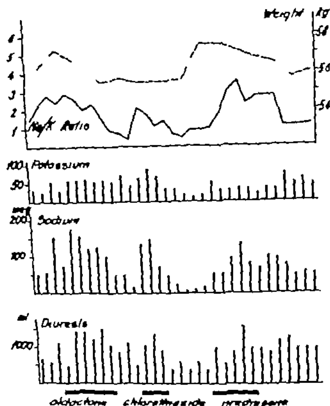


Fig. 5. Effects of spironolactone, chlorothiazide and prednisone upon the urinary output of sodium and potassium in patient with moderately decompensated heart disease (table III, case 11).

same amount of sodium, as 10 were on a low-salt diet with a daily content of about 35 mEq sodium. Sixteen were on diet having moderat salt content (about 85 mEq daily). However this does not appear to have had a decisive influence upon the response to the diuretics in our series.

No patient exhibited definite alterations in serum potassium, serum sodium, serum chloride, or total CO during the treatment periods. Similarly the potassium output was only slightly affected during the short treatment periods.

Discussion

It has frequently been emphasized that the effect of diuretics appear to be capricious and that in our present stage of knowledge regarding the pathogenesis of oedema and the mechanism of the effect of diuretics it is very difficult to foretell which diuretic is most likely to dehydrate a given patient. Even in the same patient the effect may vary. Because a given diuretic has failed at one time, it is not out of the question that it may afford a favourable effect on a later occasion. This general experience is also reflected in the present series. The explanation is presumably that oedema formation depends upon a number of different pathogenetic factors of changing mutual significance. One of the factors which have recently come into the limelight is secondary hyperaldosteronism. Increased excretion of aldosterone has been found in cirrhotic (14, 28, 33, 35)

Table IV Effects of chlorothiazide and spironolactone in hepatic cirrhosis without oedema and ascites (cases 1—3) and with oedema and ascites (cases 4—6)

Case no. Sex	Diet (mEq Na) (about)	Increase in sodium output (mEq Na)			Exch. sodium		Blood volume		Haematocrit (%)	Body weight (kg)
		Chlorothiazide	Spironolactone	Prednisone	mEq Na	mEq/kg	l	ml/kg		
1 ♀	35	—	—	—	2,550	35	—	—	—	73
		100	—	—	2,250	31	—	—	—	72
		—	400	—	2,420	34	—	—	—	72
2 ♀	85	—	—	—	—	—	—	—	—	54
		—	400	—	—	—	—	—	—	52
		350	—	—	3,210	59	—	—	—	54
		—	—	350	2,980	57	—	—	—	52
3 ♀	85	—	—	—	2 770	51	4.30	79	40	54
		300	—	—	2 470	46	4.55	84	40	54
		—	700	—	2,490	48	4 15	80	42	52
		—	—	0	2 430	46	4 15	78	45	53
4 ♂	85	—	—	—	3,940	81	—	—	—	49
		50	—	—	—	—	—	—	—	49
		—	600	—	—	—	—	—	—	45
		—	—	0	2,520	56	—	—	—	45
		—	—	—	—	—	4.26	89	28	48
		—	400	—	2,740	59	4.83	105	26	46
		—	—	100	—	—	—	—	—	—
5 ♀	85	—	—	—	2,860	60	3.88	81	35	48
		—	500	—	2,080	46	3.48	78	35	44
		100	—	—	2,140	54	3.17	81	30	39
		—	—	100	2,520	63	3.37	86	31	39
6 ♂	85	—	—	—	4,790	49	—	—	—	97
		500	—	—	4,540	48	7.20	75	39	96
		—	800	—	3,660	40	7.62	83	38	92
		—	—	0	—	—	7.50	79	39	95

Experimental data shown also in figs. 6 and 7

as well as cardiac oedema (15 34 35) although in the presence of the latter it has been more inconstant (14 19) Moreover investigations into aldosterone production (14 20 30) in oedematous patients have indicated that secondary hyperaldosteronism often co-exists at least in cirrhotics.

If a good diuretic effect of aldosterone antagonists (e.g spironolactone) implies that hyperaldosteronism is a predominant pathogenetic factor the results of Thomas and Bartter (28) suggest that in general aldosterone plays a more important role in the development of cirrhotic than cardiac oedema. The results of the present study are in keeping with this. In this connection it is remarkable that spironolactone has also been found to exert a considerable natriuretic effect in cirrhotic patients without oedema or ascites (table IV cases 1—3 and fig 6) Other

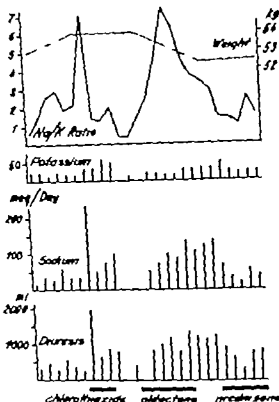


Fig. 6. Effects of chlorothiazide, spironolactone and prednisone upon the urinary output of sodium and potassium in patient with hepatic cirrhosis (table IV case 5).

uthors have also obtained an excellent diuretic effect of spironolactone used as the only agent in the treatment of cirrhosis (5 7 10, 22, 31)

Though less constantly spironolactone also had a good effect in several cases of cardiac decompensation in our series (5 out of 13) At least, our results do not give any reason to believe that the effect of this agent in sufficient doses is less reliable than that of chlorothiazide (5 out of 14) The explanation why this has not been found by others (26, 28) is possibly a generally lower dosage. The dyspepsia described by Thomas and Bartter (28) in cardiacs given large doses of spironolactone was not observed in our patients. It may perhaps be presumed that the degree of hepatic congestion in decompensated heart disease decides whether spironolactone exerts a natriuretic effect, hepatic congestion reducing the destruction of aldosterone in the liver (1 6) However we did not observe any relationship between the degree of palpable hepatomegaly and the liver function tests (thymol, bromsulphalein retention, GO transaminase) on the one hand and the effect of spironolactone on the other The above-mentioned synergism between chlorothiazide and spironolactone in cardiac patients was observed in all 3 patients treated with this combination (table II, cases 4—6)

If it is correct that the diuretic effect of prednisone is exerted by inhibition of the increase in aldosterone production a natriuretic action of this agent may be

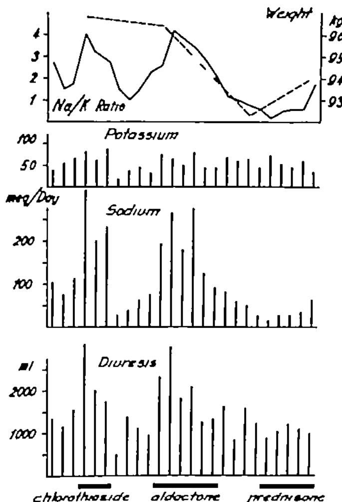


Fig 7 Effects of chlorothiazide, spironolactone and prednisone upon the urinary output of sodium and potassium in patient with hepatic cirrhosis (table IV case 6)

expected (1) when hyperaldosteronism is the predominant factor in the pathogenesis of the oedema and (2) when this hyperaldosteronism is due to a hyper production not a delayed breakdown The effect of prednisone was studied in only a small group of the present series (3 normals 5 cardiacs, and 5 cirrhotics) but our finding that prednisone was effective only in patients who also responded distinctly to spironolactone agrees with the above hypothesis. Among the cardiacs who responded to spironolactone the effect of prednisone was investigated in 4 3 of whom obtained an effect (table II cases 1 2 and table III case 11) On the other hand case 13 (table III) failed to respond to prednisone, although he had responded to spironolactone. Conversely only one of the five cirrhotics responded to prednisone. If hyper aldosteronism is due mainly to hyperproduction when associated with cardiac failure and mainly to a reduced hepatic destruction when it is associated with hepatic cirrhosis the best effect of prednisone — according to the hypothesis on the mechanism of its effect — ought to be expected in hyper aldosteronism of cardiac origin. This is also indicated by our results, but the numbers involved are too small to permit conclusions. Thomas and Bartter (28) have reported a natriuretic effect of prednisone in 2 out of 3 cirrhotic patients, but gave data for only one. According

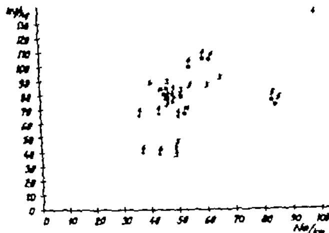


Fig. 8. Relation between exchangeable sodium and blood volume expressed per kg body weight.

● Normal.
○ Cardiac insuff.
□ Cirrhosis of the liver

The figures on the diagram refer to case numbers in tables I—IV

Abscissa: Exchangeable sodium in mEq/kg body weight.

Ordinate: Blood volume in ml/kg body weight.

to these data, the effect seems very doubtful. However the study on the rate of production of aldosterone indicates that the production is increased, at any rate in patients having hepatic cirrhosis with oedema (14, 30).

As is apparent from tables I—IV a determination of exchangeable sodium was performed in 22 cases. The normal values have been reported to range from 39 to 48 mEq Na/kg, somewhat higher in males than females (18). Tables I—IV show that per kg body weight the values, 52—97 mEq Na/kg, were increased in severely decompensated cardiacs (table II) while in the normal 3 subjects included in this determination the values were 43—48 mEq Na/kg. The moderately decompensated cardiac patients (table III) showed a minor increase (57—72 mEq Na/kg) and the mild cases of decompensation showed values from 43 to 52 mEq Na/kg (table III). Patients with cirrhosis showed elevated values, 3 without oedema and ascites from 55 to 59 and those with oedema and ascites 49—81 mEq Na/kg. During the treatment, the values fell to a varying extent, but it must be mentioned that the analytical accuracy was considerably reduced upon repeated determinations. On the whole, there was a fair correlation between the sodium output and the alteration in exchangeable sodium on the one hand and alterations in body weight on the other, although this was not invariably so (e.g. table I, case 4 and table II, case 2).

The total blood volume was determined in 13 cases. The normal values are stated to average 69 ml/kg in men and 64 ml/kg in women (2). In our normal subjects the values ranged from 61 to 67 ml/kg. In cardiac failure and hepatic cirrhosis there will be an increase in the blood volume, especially plasma volume (3, 9, 29). Among our severely decompensated cardiacs we found total blood volumes ranging from 74 to 124 ml/kg (table II), among the milder cases of decompensation 67—96 ml/kg (determined in only 2 cases). The 4 cirrhotic patients who had this test showed values of 79—89 ml/kg.

Fig. 8 gives the relation between exchangeable sodium and blood volume both per kg body weight. As might be expected, there is a relationship, an increased blood volume being associated with increased exchangeable sodium.

If exchangeable sodium is taken as a measure of the amount of extracellular fluid there was not in our series any definite difference in the ratio blood volume extracellular fluid between normal subjects, patients with cardiac and patients with cirrhotic oedema (fig 8)

Conclusions

In the present series spironolactone had a more reliable diuretic effect in cirrhotic than in cardiac oedema, while no distinct difference was found with chlorothiazide. The synergistic effect reported to be obtained by combining the two diuretics in cardiac patients was confirmed

Prednisone exerted a diuretic effect only in patients who also responded to spironolactone, and among these patients a more reliable effect was obtained in cardiac than in cirrhotic oedema

Summary

The natriuretic effect of chlorothiazide and spironolactone administered separately for a few days was investigated in 6 normal subjects 13 patients with decompensated arteriosclerotic heart disease, and 6 patients with hepatic cirrhosis, the latter including 3 with oedema and ascites.

Among the decompensated cardiacs chlorothiazide was effective in 5 out of 14 spironolactone in 5 out of 13 Among the cirrhotics chlorothiazide was effective in 3 out of 6 and spironolactone in 6 out of 6

In 5 of the decompensated cardiacs and in 5 of the cirrhotics, we also studied the effect of prednisone upon the sodium output. In the cardiacs prednisone exerted a natriuretic effect in 3 and among the cirrhotics in 1 In cases where prednisone was effective, spironolactone was also effective.

Determinations of exchangeable sodium and of blood volume were carried out in 22 and 11 cases respectively Both were found to be elevated per kg body weight in severely decompensated cardiacs as well as in the cirrhotics A relationship was found between these two parameters, expressed per kg body weight — an increased blood volume being as might be expected associated with an increase in exchangeable sodium.

Acknowledgement

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An Ornithosis Epidemic in Örebro

By

K. ALESTIG, K. BAKOS, J. BARR and L. HELLER

In the autumn of 1960 thirteen serologically verified cases of ornithotic pneumonia were observed in the town of Örebro. The disease has received scant attention in Sweden, where the above cases constitute the largest epidemic of its kind yet recorded.

Ornithosis has been known since the closing years of last century. It was formerly referred to as psittacosis because various members of the parrot family carry the infection. The designation ornithosis has become increasingly common since the identification, in a hundred or so species of birds, of virus strains belonging to the *Miyagawanella psittaci* group. Although the two terms are usually considered synonymous, certain authors would prefer to limit the term psittacosis to the perhaps more serious infections from birds of the parrot family.

In 1892 and 1929 several countries had large-scale epidemics of ornithosis due to the introduction of infected parrots from South America. Around the time of the 1929 epidemic the disease was observed for the first time in Sweden, where Johnsson (13) in 1930 described three cases of pneumonia in seamen who had brought home diseased parrots. Serum diagnosis of the condition was not yet available; not until 1939 was the first serologically verified case of ornithosis reported by Rune Frisk (7).

The cases of ornithosis which occurred in the Faroe Islands (5) in the nineteen-thirties, and which were due to infection from arctic fulmars, are well known. Over a five year period 68 persons acquired the disease, with a mortality of 22 per cent. The hunting of these birds was prohibited and no further cases were reported.

The incidence of ornithosis appears to have risen substantially during the last decade. In the late nineteen-forties the annual rate noted in the U.S.A. was 25 to 35 cases; in the period 1954—1956 it amounted to 400—500 cases. Factors contributing to the apparent rise may be increased commerce in birds, relaxation of the previously stringent trade restrictions, and improved diagnostic facilities. In the U.S.A. today ornithosis presents a major problem in e.g. the turkey industry

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In Sweden on the other hand the number of cases has remained low In 1958 four cases were reported from Stockholm, the infection being traced to sick, virus-carrying parrots (4 15) In 1955 Grubb (8 9) reported three cases in one family of pigeon breeders. Of 114 sera sent to the Bacteriology Department of Lund University for cold agglutinin titration because of pneumonia 20 showed positive complement fixation tests for lygranum antigen and six of these 20 cases had symptoms suggestive of clinical ornithosis. At the National Bacteriology Laboratory Stockholm Heller (10) in 1955—1956 diagnosed in a similar series 28 cases of ornithosis from different parts of the country

In Denmark and Finland extensive investigations of ornithosis have been conducted during recent years by Volkert (18) and Jansson (12) They noted an ornithosis incidence of approximately 5 per cent in major pneumonia series and a high incidence of virus infection in the pigeon populations of Copenhagen and Helsinki Pigeons, it may be added have been regarded as a possible source of human ornithosis

In reports from Norway and earlier from the U.S.A the theory has been advanced that there are human-adapted strains of the ornithosis virus which may be spread from person to person (5 6 11)

The most extensive treatise yet published on ornithosis is that of Beaudette et. al. (1)

Course of the epidemic

In the autumn of 1960 the Infectious Diseases Hospital at Örebro admitted a number of patients with pneumonia characterized by high sedimentation rates, low white blood counts, and a protracted, intermittently severe course. Somewhat fortuitously we screened paired sera from one patient against lygranum antigen, and rising titers thereby disclosed ornithosis. In this patient's home there was a canary which we examined for ornithosis virus with negative results. Many of the other patients had no knowledge whatsoever of contact with birds. Since, however our suspicions had been aroused we tested sera from patients previously hospitalized for pneumonia and thereby discovered no fewer than ten additional cases. In other words, the epidemic came to light retrospectively

All three pet shops in the town were for various reasons, under suspicion. In consultation with the local health authorities the shops were closed and disinfected and their bird stocks destroyed. Through announcements in the local press, particulars of some three hundred bird owners and their pets were obtained. Birds from the pet shops and from private owners were subjected to virus isolation tests, as were local pigeons. All personnel from the three shops as well as approximately two hundred bird owners and a like number of controls were tested for titers against lygranum antigen, a further two cases of ornithosis being thereby detected.

The series thus totalled 13 cases of verified ornithotic pneumonia. The first patient had developed symptoms at the end of October 1960 the last one in early January 1961 when the field investigations were instituted.

Material and methods

Virus isolation

The three pet shops housed a total of 190 birds, all of which were destroyed and 36 sent for virus isolation tests. In the town of Örebro, population approximately 75,000, a total of 650 cage birds were reported in private ownership. Sixty-five of them were destroyed — mostly at the owners request — and 36 selected for virus isolation tests. All such tests were carried out at the National Institute of Veterinary Medicine, Stockholm, where Bakos in 1958 had isolated the ornithosis virus from pigeons, using the following procedure.

After necropsy three 10 % suspensions were prepared from respectively the liver and spleen, the lungs, trachea and nose and the

Table I. Virological examinations of 72 birds

Owner	Birds	Symptoms	Virus isolat.	Comments
Pet shop N. Z.	7 canaries	–	–	13 cases of human ornithosis
	2 finches	–	–	
	3 parakeets	–	+++	
	1 parrot	–	+	
Pet shop O.	5 parakeets	–	–	No human cases
	3 finches	–	–	Proprietor serologically negath
	2 canaries	–	–	
Pet shop T	9 parakeets	–	–	N human cases
	4 canaries	–	–	Proprietor serologically negath
U.B.W ♀ 20	1 finch	+	+	Purchased at T Dec. 60
	2 finches	1 died	–	Owner serologically negative
C.H., ♀ 64	1 parakeet	Died	+	Purchased at T Dec. 60 Owner serologically negative
Child psychiatry Department	2 parakeets	1 died	++	No human cases
Other private owners	30 of various species	+–	–	No human cases

brain, with the use of phosphate buffer solution (pH 7.4) and sterile sand. The suspensions were left to stand overnight at +4 C, then centrifuged for 15 min t 3,000 p.m. To the supernatant was added streptomycin 1.0 mg/ml. Where the brain suspension, on bacteriological examination, proved to be non-sterile penicillin 10,000 I.U /ml was added Suspensions from each bird were then used to infect three white mice (weight 14—16 g) intraperitoneally (0.5 ml of the liver-spleen suspension), intranasally under ether anesthesia (0.05 ml of the lung-trachea-nose suspension), and intracerebrally (0.03 ml of the brain suspension) After eight days observation the mice were killed and necropsied. From the liver spleen, lungs and brain 10 suspension was prepared as described above and, after bacteriological examination, was used for second mouse passage via intraperitoneal and intranasal inoculation. At the end of eight days the mice were killed, after which third mouse passage was done as before.

I positive instances the mice developed symptoms (lassitude anorexia, spiky coat, ey discharge) four to eight days after inoculation, and several of them died. Necropsy revealed serofibrinous peritonitis, fibrinous perihepatitis and perisplenitis with enlarged liver and spleen, and pneumonia. Some of the second and third passage mice, though to all appearances quite healthy nevertheless exhibited relatively mild lesions when killed and necropsied eight days after inoculation. Smears from the suspect organs were stained by the methods of Giemsa and Castaneda. In positive instances intra- and extracellular elementary bodies were microscopically de monstrable. The strains isolated via mouse passage multiplied in a fertile hen's egg (inoculated into the yolk sac) and after embryonic death (five to eight days after inoculation) elementary bodies were de monstrable in the extra-embryonic membranes. The strains cultivated in eggs were pathogenic for white mice.

The results of the virus isolation tests are set forth in table I

Virus-carrying birds were detected in the stock from pet shop NZ but not in that from the other tw shops. Although the birds, according to the proprietor had appeared healthy it may be assumed that virtually the

In Sweden on the other hand the number of cases has remained low In 1953 four cases were reported from Stockholm the infection being traced to sick, virus-carrying parrots (4 15) In 1955 Grubb (8 9) reported three cases in one family of pigeon breeders. Of 114 sera sent to the Bacteriology Department of Lund University for cold agglutinin titration because of pneumonia 20 showed positive complement fixation tests for lygranum antigen and six of these 20 cases had symptoms suggestive of clinical ornithosis At the National Bacteriology Laboratory Stockholm Heller (10) in 1955—1956 diagnosed in a similar series 28 cases of ornithosis from different parts of the country

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Course of the epidemic

In the autumn of 1960 the Infectious Diseases Hospital at Örebro admitted a number of patients with pneumonia characterized by high sedimentation rates, low white blood counts, and a protracted, intermittently severe course. Somewhat fortuitously we screened paired sera from one patient against lygranum antigen, and rising titers thereby disclosed ornithosis. In this patient's home there was a canary which we examined for ornithosis virus with negative results. Many of the other patients had no knowledge whatsoever of contact with birds. Since, however our suspicions had been aroused we tested sera from patients previously hospitalized for pneumonia and thereby discovered no fewer than ten additional cases. In other words, the epidemic came to light retrospectively

All three pet shops in the town were, for various reasons, under suspicion. In consultation with the local health authorities the shops were closed and disinfected and their bird stocks destroyed. Through announcements in the local press, particulars of some three hundred bird owners and their pets were obtained. Birds from the pet shops and from private owners were subjected to virus isolation tests, as were local pigeons. All personnel from the three shops as well as approximately two hundred bird owners and a like number of controls were tested for titers against lygranum antigen, a further two cases of ornithosis being thereby detected.

The series thus totalled 13 cases of verified ornithotic pneumonia. The first patient had developed symptoms at the end of October 1960 the last one in early January 1961 when the field investigations were instituted.

Material and methods

Virus isolation

The three pet shops housed a total of 190 birds, all of which were destroyed and 36 sent for virus isolation tests. In the town of Örebro, population approximately 75,000, a total of 650 cage birds were reported in private ownership. Sixty-five of them were destroyed — mostly at the owners request — and 36 selected for virus isolation tests. All such tests were carried out at the National Institute of Veterinary Medicine, Stockholm, where Bakos in 1958 had isolated the ornithosis virus from pigeons, using the following procedure.

After necropsy three 10 % suspensions were prepared from respectively the liver and spleen, the lungs, trachea and nose, and the

Table III. Titers against lygranum antigen

No.	Group	<1/5	≤1/10	1/20	1/40	1/80
196	Owners of birds	179	14	1	1	1
38	Hospitalized patients	31	5	2	—	—
41	Miscellaneous[3]	34	7	—	—	—
200	Controls from the surg. outpat. Dept.	182	15	3	—	—
Total 475	—	426	41	6	1	1

Radiologically verified pneumonia.
Fevers of various origin.
Hospital staff, health officers etc.

ornithosis and lymphogranuloma venereum could be confused.

All sera were titrated also against an ornithosis antigen obtained from the National Serum Institut in Copenhagen. No major disparities in titer strength were noted, and for practical purposes it is probably immaterial which antigen is used, although according to some authors the ornithosis antigen gives somewhat higher values (2).

Each of the 13 cases of ornithotic pneumonia was verified by complement fixation reactions against lygranum and ornithosis antigen. All 13 clinical cases showed, as is evident from table II, at least a fourfold rise or fall of the titers against lygranum antigen or else, as in the last two cases, titers of at least 1 40 which, along with the clinical symptoms, may be considered to have established the diagnosis. It should be made clear that the rising titers sometimes came late in the course of the disease, that the maximum was often reached in the second to third month, and that high titers persisted for up to a year or even longer. One conceivable explanation of the long persistent titers might be sought in incomplete antibiotic cures.

Since no extensive investigations of ornithosis had been conducted in Sweden, sera from some two hundred bird owners and a like number of controls were screened. The controls were randomly selected patients attending the surgical outpatient department of Orebro Hospital. Both groups were questioned as to known sickness of either humans or birds in their environment, and as to any contact with pet shops.

Table III shows the incidence of titers against lygranum antigen in various groups. In the bird-owner group we noted only two high titers — 1 40 and 1 80 respectively. Since these serum samples were taken five and eleven months respectively after the onset, and since both patients had had pneumonia and contact with pet shop NZ during the epidemic, they are included in the clinical series (the last two cases in table II).

Otherwise no conspicuous inter-group differences were noted. Approximately 10 % of both bird owners and controls had positive, though low titers — but this proportion is quite common in investigations of this type (5, 14). Of the 200 controls 18 had had contact with birds or bird shops, but in each case the titers were below 1 5.

The results which emerge from tables II and III suggest that high titers of complement-fixing antibodies against lygranum or ornithosis antigen constitute reliable evidence that clinical ornithosis has been present. The observed low titers showed no correlation to clinical symptomatology or contact with birds.

Clinical considerations

The symptomatology was, without exception, of the classic type, with radiologically verified bronchopneumonia or pleuropneumonia and, in some cases, severe impairment of the general condition. The course was protracted, the patients being unfit for work for an average of two months. As regards laboratory tests, all cases observed had

Table II Titers against lygranum antigen Time after onset

Pat. Sex Age (yrs)	Days				Months											
	0—5	5—10	10—20	20—30	1—2	2—3	3—4	4—5	5—6	6—7	7—8	8—9	9—10	10—11	11—12	12—13
K. M. ♂ 39	<5	10	80	—	—	—	—	—	—	—	—	—	—	—	—	—
S. E. ♀ 30	—	5	20	40	—	—	40	80	—	—	80	—	—	—	40	—
M. N ♀ 42	—	5	—	—	40	—	—	20	—	—	20	—	—	10	—	—
G H ♀ 47	—	—	20	—	640	—	320	—	—	—	—	—	80	—	—	—
K. H ♂ 49	—	—	<5	—	20	—	160	—	40	—	—	—	20	—	—	—
E. P ♂ 38	<5	—	—	20	80	80	—	—	—	—	—	—	80	—	—	—
H G ♂ 30	—	—	—	—	160	160	80	40	—	—	—	—	—	40	—	—
K. E. E. ♂ 32	—	—	—	—	160	160	—	—	—	—	80	—	—	—	80	—
K. Å. E. ♂ 39	—	—	—	—	—	80	80	—	—	40	—	—	—	20	—	—
V L. ♂ 52	—	—	—	—	40	—	—	—	10	—	—	—	5	—	—	—
M. G ♀ 56	—	—	—	—	—	—	40	—	—	—	10	—	—	—	10	—
O O ♂ 61	—	—	—	—	—	—	—	—	80	—	—	—	—	—	—	—
P B. ♂ 13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	40

entire bird stock of pet shop NZ was infected since in avian ornithosis there is often little or no outward evidence of disease.

It is worthy of note that two private owners had virus-carrying birds which had been purchased in pet shop T during the epidemic. It is not unusual, however, to find virus-carrying birds and no cases of human ornithosis in their environment. If, on the other hand, the general condition of the birds deteriorates or the number of mildly infected young birds in the stock increases, human cases may suddenly appear. Differing degrees of virulence of the virus strains, or intensification of their virulence, may also be factors in spread of the infection. In this epidemic it was clearly established that each of the 13 patients with clinical ornithosis had been in contact with pet shop NZ prior to the onset, as will be recounted in detail later on (table IV).

Surprisingly enough, two caged parakeets kept at the Child Psychiatry Outpatient Department of Örebro Hospital proved on examination after the sudden death of one of them, to be virus carriers. No verifiable clinical cases linked with these birds were detected.

Virus isolation tests of specimens from human ornithosis cases were not general since all patients were in the convalescent stage when the nature of the epidemic became evident. A few isolation tests were done with feces and sputum, but the results were negative. This, indeed, was to be expected inasmuch as such specimens, like blood samples, should be taken during the acute stage of the disease. There are, however, occasional recorded instances of positive sputum tests some years after the disease (1).

The ornithosis virus, as pointed out above, has been demonstrated in the pigeon populations of several European cities — and these birds are regarded as a possible source of human ornithosis (17). Thirteen pigeons from the town of Örebro were therefore tested for virus, but with negative results.

Serological investigations

The ornithosis virus is one of a group which includes the etiologic agents of lymphogranuloma venerum and trachoma. All of these strains have certain antigens in common, so that antigens from different strains can be used in the diagnosis of ornithosis. Antigen prepared from the lymphogranuloma venerum virus is customarily employed, as in our cases (Lygranum, Squibb). It is scarcely possible that two diseases so clinically divergent as

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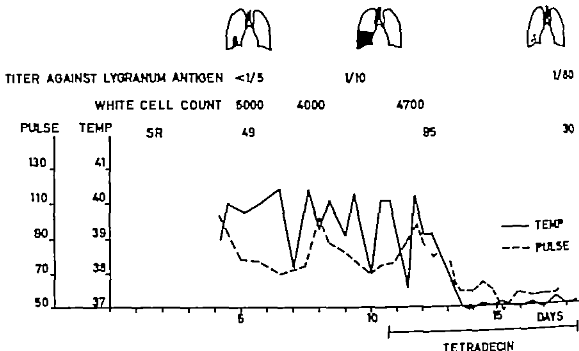


Fig 1 K. M. ♂ 39

high sedimentation rates (mean 85 mm) low white cell counts (mean 5,500/mm³) and negative cold agglutination tests. Differential white blood counts showed, on the whole, normal values. The pulmonary radiograms disclosed both lobar pneumonia and bronchopneumonia and pleuropneumonia. Here follows a typical illustrative case

On November 23 1960 a 39-year-old German dental surgeon paid a single visit to pet shop NZ and 11 days later developed remittent hyperpyrexia and headache. On the fourth day after the onset he became stuporous and was admitted to the Infectious Diseases Hospital at Örebro with suspected meningitis. Lumbar puncture did not corroborate the diagnosis, but chest radiograms revealed pneumonia. The following day the patient had high fever and repeated chills. Sepsis was suspected, but blood culture was negative. Since further pulmonary radiograms indicated progression, treatment with Tetradecin was instituted. Two days later the patient was afebrile and considerably improved. He was discharged after two weeks hospitalization He owned a canary that was subsequently examined virologically with negative results. Sera from members of his family who had not visited the pet shop showed negative antibody titers against lygranum antigen.

In common with the above case the majority of pneumonia cases were initially regarded as virus pneumonia, and hence many of them did not receive antibiotic treatment. In those cases where broad spectrum antibiotics were given, the patients became afebrile within two or three days. It has in fact been pointed out that when a virus pneumonia responds favorably to such antibiotics ornithosis should be suspected (16) The fact that broad spectrum antibiotics were so little used may perhaps have contributed to the persistence of comparatively high titers against lygranum antigen (table II)

Mode of spread of the disease

Of the three pet shops in Örebro, only NZ was found to have virus-carrying birds (table I) In each of the 13 cases of human ornithosis this pet shop was implicated, as shown in table IV which also gives certain other data of relevance to these patients.

The infection appears to have been acquired solely via direct contact with pet shop NZ With

Table II. Epidemiological data

Pat. Sex Age (yrs)	Visits to pet shop N.Z.	Visits to other pet shops	Owner of birds	Virus isolation in birds	Comments
R. E. E. ♂ 32	+++	–	++	++	Proprietor of N.Z.
H. O. ♂ 50	++	+	++	++	Shopassistant t N.Z.
R. E. ♀ 30	–	–	+	+	Wife of proprietor
K. M. ♂ 59	Once	–	+	–	Family members serologically negative
M. N. ♀ 42	Once	–	+	Not examined	Family members serologically negative
G. H. ♀ 47	+	+	– aquarium	–	—
K. H. ♂ 49	+	–	– aquarium	–	—
E. P. ♂ 58	+	–	– aquarium	–	—
M. O. ♀ 56	+	–	–	–	—
K. A. E. ♂ 39	Twice	–	– aquarium	–	—
V. L. ♂ 52	Twice	+	+	Not examined	Family members serologically negative
O. O. ♂ 61	+	–	+	Not examined	—
P. R. ♂ 13	+	–	– aquarium	–	—

but one exception, all patients had visited this shop 6 to 15 days before the onset — several of them only once or twice. The exception was the wife of the proprietor (case 3) who disliked the shop and never set foot there. I all probability she was infected by the shop only parrot, which harbored the virus and which had been kept for two days in the family bedroom. *Six patients were not bird owners, and fi of them had visited the pet shop only to buy food for their quarium fish.* It was then necessary when interviewing the patients, to ask whether they had aquaria.

I is noteworthy that with the exception of case 3 no evidence was found of spread of the infection via sick birds in private ownership. Yet brief visit to pet shop NZ sufficed for contraction of the disease. The corollary is that this was an extremely virulent strain of virus, as is also evidenced by the severity of the clinical symptoms. Indeed, i is possible tha the disease was "true psittacosis" stemming from the above-mentioned virus-carrying parrot. This could perhaps account for the fact that no other virus-carrying birds were found among those sold during the relevant period these birds were canaries and finches which had been in the shop for only ery short time.

It was not possible to ascertain by what means pet shop NZ had been infected. Its psittacine birds had been imported via Landskrona, where a bird quarantine is in force. Although no suspected ornithosis infections were reported from that seaport, it is known that infected Amazon parrots had been imported there in 1957 and that, despite the quarantine, they had caused t least five cases of human psittacosis, one of them fatal (19). I addition to its imports pet shop NZ procured non-psittacine birds to large extent by exchange deals.

No cases of human ornithosis secondary to the 13 in this series were observed.

Discussion

The epidemic described here was discovered by chance and comprised 13 verified cases of human ornithosis. It is possible that many other cases were implicated since non-hospitalized patients with pneumonia are often treated with broad spectrum antibiotics. The epidemic came to light retrospectively and might easily have passed unnoticed. About one

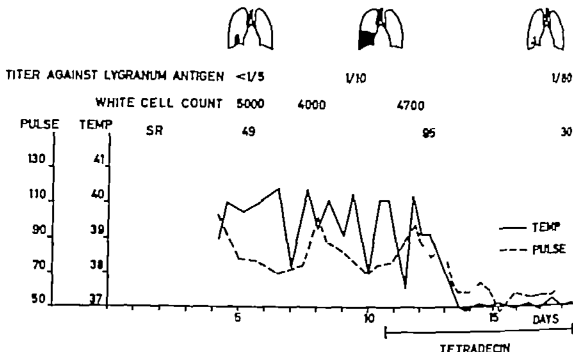


Fig. 1. K. M. ♂ 39

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half of the patients were not bird owners but acquired the disease when visiting an infected shop — a fact which amply illustrates the importance of detailed case histories for elucidation of the mode of spread in ornithosis infections. The infectiousness appeared to be very high. Ornithosis virus was not demonstrable in the local pigeon population.

No large-scale investigation into the incidence of ornithosis associated with pneumonia has yet been conducted in Sweden. Investigations in Denmark and Finland, as mentioned in the introduction, have shown ornithosis to be a cause of more than 5 per cent of all pneumonia cases. Undiscovered epidemics of the aforementioned type may to a certain — perhaps large — extent account for this incidence. If so, measures to control ornithosis should be instituted particularly against cage birds — and chiefly in connection with importation.

The present regulations governing the importation of birds into Sweden afford no guarantee against the introduction of virus-carrying birds. Parrots and parakeets are subject to two months quarantine and sick birds are destroyed. Other species of birds are merely inspected, and for individual birds directly imported by the owner there is no control at all. In view of the fact that infected birds may appear quite healthy the present restrictions are of course inadequate. For small birds serum diagnosis is not yet practicable, and even for larger birds it presents formidable difficulties. Treatment of bird food with broad spectrum antibiotics would conceivably be of benefit in terms of prophylaxis. In Sweden, it may be added, human ornithosis is a reportable disease subject to the provisions of the Epidemic Diseases Act.

Although ornithosis in poultry farmers has heretofore presented no problem in Sweden, two cases have in fact been detected in employees of large poultry farms since this manuscript was prepared. In the future, ornithosis may perhaps come to be as great a problem here as it is in the U.S.A., where it is estimated that eradication of the approximately 400 cases of human ornithosis annually would require control of a bird population totalling around 10 millions.

Summary

A local epidemic comprising 13 verified cases of ornithosis in the town of Örebro, Sweden, is reported. The epidemic was discovered by chance after the event, and might easily have passed unnoticed.

On detailed recording of the case histories the infection was found to stem from a single pet shop which all of the patients had visited — several of them just once. Approximately one half of the patients were not bird owners but had visited the shop to buy food for their aquarium fish.

The last two cases were detected one year after the epidemic when a large number of persons who had been in contact with birds, as well as a like number of controls, were screened. No ornithosis was demonstrable in the pigeon population of Örebro.

Investigations conducted in Denmark and Finland have implicated ornithosis as the cause of more than 5 per cent of all pneumonia cases — an incidence which may be partially attributable to similar undetected epidemics.

Possible measures to prevent the spread of ornithosis infection are discussed.

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From the Medical Service I Sahlgren Hospital, Gothenburg, Sweden

Serum Lipids During the Course of an Acute Myocardial Infarction and one Year Afterwards

By

Gösta Tibblin and Kim Cramér

Much attention has been focused on the relationship between serum lipids and the incidence of myocardial infarction. Conclusions have been drawn from examinations carried out soon after the onset of an acute myocardial infarction although there has been no follow-up for any length of time.

Changes in serum cholesterol immediately following the onset of an infarction were reported by Welin in 1948 (19) and later on by Biörck et al. 1957 (3) and Björntorp and Malmcrona 1960 (4). A thorough study was carried out by Dodds and Mills in 1959 (10) who determined changes within the serum lipoproteins with varying techniques, as well as movements in serum cholesterol.

The aim of this investigation was to complete the study of serum lipids, to determine serum glycerides and serum phospholipids during the first weeks after an acute myocardial infarction, and to establish the stat of serum lipids three months and one year after the acute onset. Furthermore, we wanted to check glucose tolerance and thyroid metabolism.

Submitted for publication April 1 1963.

Methods

Serum cholesterol was determined according to Theorell and Widström (17) as modified by Cramér and Isaksson (8) phospholipids according to Svanborg and Svennerholm (16) serum glycerides as glyceride glycerol according to Carlson and Wadström (5) modified by Carlson (6). Peroral glucose tolerance test was performed according to Luft and Goldberg (two doses — one hour test). Determination of protein-bound iodine was made according to Acland (1).

Material

All patients below the age of 70, who were admitted to the First Medical Service of Sahlgren's Hospital with the diagnosis of acute myocardial infarction were included in the study. The diagnostic criteria were the conventional ones: typical chest pain, ECG-changes, leukocytosis, increase in serum GOT level and fever. No heparin was given, but all patients received dicoumarol therapy during the first three weeks of the disease. 69 patients were admitted between June 1st 1960—January 31st 1961 during which time the study was performed. The only selection which was made was that the onset of the disease must not have taken place more than 24 hours before admission. Blood specimens were drawn im-

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mediately after admission and then in the fasting state on the second, seventh, fourteenth, and 21st day of the hospital stay. Serum lipids, glucose tolerance, and thyroid metabolism were examined 3 months later. A complete series of specimens was obtained in 24 patients. Twenty-two of these (17 men and 5 women) were reexamined one year after they had left the hospital. Two patients had died in the meantime. Three of the men had suffered from previous infarctions. The mean age of this group was 54 years. Incomplete series of specimens were obtained from 43 patients. These results were discarded and no longer used in this study. The mean age of this group was 58 years. There were no differences between the "complete" and the "incomplete" groups as regards sex distribution, social state, frequency of diabetes, hypercholesterolemia, hypertension and/or severeness of the disease.

All patients were kept on a fat-restricted diet during their hospital confinement with 15 to 20 % of the calories as fat.

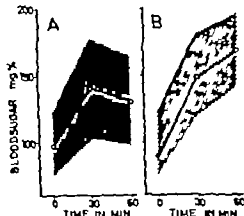


Fig. 3. Glucose tolerance test according to Goldberg and Luft (12) in 27 survivors of myocardial infarction, 3 months after the acute episode.

The left diagram represents the normal material of Goldberg and Luft ± 2 S. D. (the shaded area). The right diagram represents the present material ± 2 S. D.

Results

The changes of serum cholesterol and phospholipids were uniform. The percentage changes from the initial level are given in fig. 1. Both decreased during the first week after the infarction; the difference was highly significant ($p < 0.001$) on the seventh and the fourteenth day. The initial level was again attained after three weeks. No further changes were seen.

The glyceride-glycerol values showed a drop from the first day to the second. This was probably caused by the fact that the first day specimen was not withdrawn while the patient was fasting. All calculations ha[ve] therefore been made with the second day value as a basis, and after logarithmic conversion of the values because of a skew distribution. The [a]bsolute values are given in fig. 2. The values rose from the second day and culminated after three weeks whereupon they declined and reached the initial level after 12 months. The elevations after three weeks and after three months were significant $p < 0.001$ and < 0.01 respectively. In [a] total of 31 patients, basal metabolic rate, serum protein bound iodine and the thyroid uptake of I^{131} were performed three months after the acute episode. All results were within normal limits.

The glucose tolerance test was performed in 27 patients without known diabetes.

The normal limits given by Goldberg and Luft (12) are shown to the left in fig. 3 with the shaded area representing ± two standard deviations. The results from the 27 patients in this study are given to the right in fig. 3. The mean value of the post-infarction patients was higher than the mean value of Goldberg and Luft ± two standard deviations. A definitely lowered glucose tolerance was found in 1[.] of the 27 patients. None had glucosuria in connection with the test.

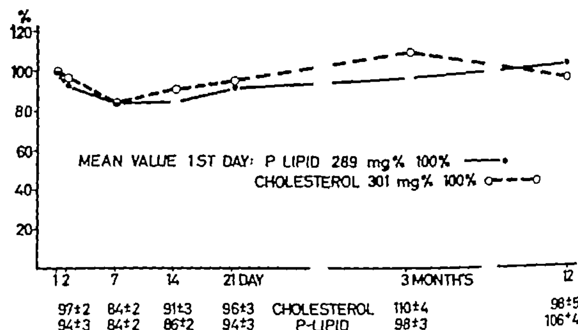


Fig 1 Changes in serum cholesterol and phospholipids during the course of an acute myocardial infarction and 3 and 12 months after onset in 22 patients. The values are expressed as % of the original level and ± S. E. of mean.

The values for the seventh and the fourteenth day are significantly lower ($p < 0.001$) than the values at admission.

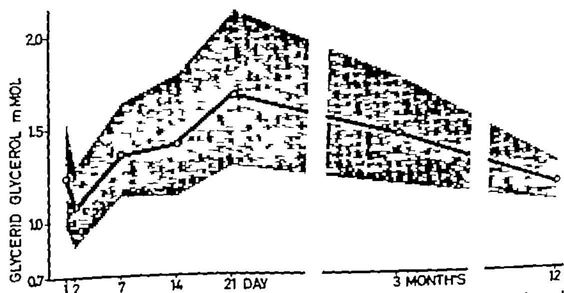


Fig 2. Changes in serum glyceride-glycerol during the course of an acute myocardial infarction and 3 and 12 months after onset in 22 patients. The shaded area represents ± 1 S. D. The values were transformed into logarithms before statistical calculations. The values from the twenty-first day and three months later are significantly different ($p < 0.001$ and < 0.01 respectively) from the values on the second day (the specimens withdrawn at admission were not obtained while the patients were fasting).

who returned to their former dietary habits and those who changed their diet. None of the patients were on rigid fat-restriction one year after the infarction.

Summary

In 24 patients with acute myocardial infarction, serum cholesterol and phospholipids showed a significant drop during the first week but returned to the initial level after three weeks. The serum glyceride-glycerol rose to a maximum after three weeks. All serum lipids had returned to their initial level after 12 months. Care must be taken when serum lipids are evaluated, so that the influence of the acute attack is abolished.

Examination of the thyroid function in survivors of myocardial infarction disclosed no abnormalities.

A decreased glucose tolerance after glucose given perorally was seen in 14 of 27 survivors of myocardial infarction. None of them had clinically overt diabetes, and none showed glycosuria during the test.

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Discussion

The variation of serum cholesterol values agrees well with the findings of Welin (19) and of Dodds and Mills (10). The similar change within the phospholipids could be predicted but has not been described earlier. This is also the case with the changes in the glyceride-glycerol if the augmentation of the Sf 20—400 lipoproteins, found by Dodds and Mills 1959 is considered. Nicolaysen and Westlund (15) have recently made the same observation concerning the changes in glyceride glycerol. Some evidence emphasizes the importance of the serum glycerides in the genesis of myocardial infarction. They might therefore be of predictive value for myocardial infarction (2, 7). It is, however, of utmost importance that the specimens are withdrawn after a considerable period of time from the onset of the infarction. More than 50 % of Albrink's (2) specimens were examined within one month after the acute episode which probably influences the level of glycerides. Carlson's material is more reliable in this respect but his glyceride values are higher than ours on the second day or 12 months after the onset. This may be due to different levels in patients from different parts of Sweden as indicated earlier (9, 16).

It is known that the serum urinary excretion of noradrenalin rises during the course of an acute myocardial infarction (11) and that the eosinophil count drops (14). Catecholamines cause an elevation of free fatty acids in serum (13). It seems reasonable to attribute the lipid changes during the course of an acute myocardial infarction to an increased output of adrenergic substances in the acute phase of the disease. It is more difficult to explain the elevation of serum glycerides as long as three months after the onset. A decreased glucose tolerance after glucose given intravenously has been described by Wahlberg (18) who examined nondiabetic patients with atherosclerotic disease in the heart and/or the extremities. The present investigation also emphasizes the view that myocardial infarction is not solely connected with a disturbed lipid metabolism.

The objection could be raised that the lipid changes are not necessarily due to the infarction but may have occurred before the infarction and virtually may have been the cause of the heart attack. The drop in serum cholesterol and phospholipid would then reflect a return to the patients' ordinary level rather than being secondary to metabolic disarrangements in connection with the disease. There is, however, no evidence at present to support such an interpretation.

The patients of the present study were kept on a fat restricted diet containing 15—20 % of the calories as fat, during their stay in hospital. The same diet was given to 10 control patients, and the serum lipids were studied before and seven days after the diet was given. There were insignificant drops in serum cholesterol and phospholipids and insignificant rises in serum glycerides.

When the patients were dismissed from the hospital they were told to avoid large meals and to be careful with the use of fat in the diet but they were not instructed to add polyunsaturated fat to the diet. Twelve of the patients did not follow these instructions but kept their former dietary habits with about 40 % of the calories as fat. Ten patients obeyed instructions more or less carefully and two added polyunsaturated fat to the diet. There was no difference in the future course of the serum lipids between the 12

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Bilateral Primary Renal Carcinoma?

By

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Hypernephroma is a rather common form of malignancy. Its way of metastasizing is well known. Bilateral, true primary hypernephroma in adults is rare, whereas in children double embryonal adenomyosarcoma of the kidney (Wilms tumour) is not infrequent (4). Some of the cases earlier reported are perhaps examples of unilateral renal neoplasms giving metastases to the other kidney. A case of bilateral, possibly primary renal carcinoma is reported.

Case report

A 48-year-old nullipara admitted to the Department of Medicine of Sala Hospital in August 1962. Details of the previous history are uncertain due to the considerable mental retardation of the patient. Since 1946, she had been patient at a mental hospital. The last few years she had complained of some episodes of "cystitis". An abdominal mass was then discovered and the patient was transferred to the Department of Medicine, where the author was working temporarily. On examination the debility and cachexia were well recognizable. There were no signs of cardiac decompensation. Blood pressure 160/90. Normal body temperature. A few rhonchi were heard on both sides of the lungs. A large, fixed, hard and nodular mass, insensitive to pain, was felt in the abdomen on both sides. The bilateral masses extended from the costal margins to the iliac fossae. The left mass was somewhat larger exceeding the midline a little. There were no abnormal findings in the fundus oculi.

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NPN 27 and serum creatinine 0.7 mg %. ESR 29—54 mm/hour. Liver function tests were normal. Total plasma protein 7.2 g %. α_2-globulin 1.1 g %. Hb 12.2 g %. There was constant macroscopical haematuria and albuminuria, Esbach 0.1—0.4 ‰. Specific gravity of the urine was 1.027 at the most. X-ray examination of the chest and the lungs was normal except for many pepper corn-sized calcifications at both apical regions and in the right hilar region.

On intravenous pyelography the contrast medium was visible at a normal time on both sides. The calyces and the pelvis were very much dilated on the left side. A more detailed examination of the renal pelvis or the ureters on both sides, was, however, not possible due to deficient contrast filling (fig. 1).

Renal arteriography showed a large swelling in the lower part of the left kidney with small areas of calcification. There was considerable curved deviation of the intrarenal vessels from this swelling. At least three smaller swellings were visible in the upper left pole. In the upper pole of the right kidney a large swell-



Fig. 2.) Left renal angiogram. The large swelling in the lower part of the kidney is visible b) Right renal angiogram. In the upper part the hepatic artery is visualized and below the right renal artery and the renal swelling are seen.

liver and cerebrum. The tumours spread via the renal veins or via the lymphatics. A pyelorenal reflux may also mediate a tumour spread in instances of increased pressure of the urinary tract (10). There are numerous collaterals between the caval veins and the veins of the vertebral column, pelvis and retroperitoneal space thus giving many possibilities of metastatic spread. Direct metastasis from a renal neoplasm to the opposite kidney is uncommon. Metastatic ureteral involvement from a renal adenocarcinoma of the same or contralateral kidney (8, 12) or bilateral papillary carcinoma of the ureters or the pelvis is even rarer (3, 9). Bastable has made a review of twenty earlier cases of bilateral carcinoma of the kidneys (2). Several of these accepted cases may have been instances of metastases to the other kidney.

The question of the renal tumours being primary or secondary is difficult to decide. Billroth postulated in 1879 that to be considered primary each tumour must have a different point of origin, different pathological structure and produce its own metastases. These requirements are, however, too strict. Other factors in favour of independent bilateral renal



Fig 1 Intravenous pyelogram. Note the dilatation of the calyces and the pelvis on the left side. A large left-sided swelling is just discernible

ing was found and a smaller swelling was seen in the central part of that kidney On both sides there was rather thin filling of contrast medium of the remaining renal parenchyma. It is doubtful if there was any pathological filling of the swellings or not. The border between the swellings and the renal parenchyma was, however not sharp (fig 2)

Exploratory laparotomy was performed on August 30 through an incision in the left iliac region. The left kidney was largely replaced by a tumour mass the size of a child head. The tumour was lobular and bright-coloured The largest excrescence was situated in the lower part. There was no tumorous engagement of the renal capsule, the renal vein or the peritoneum. The right kidney was somewhat smaller It was invaded by a lobular tumour of similar appearance to the left tumour The largest excrescence was situated in the upper part. There was no apparent infiltration of the capsule or the renal vein. A walnut-sized biopsy was taken from the left tumour

Histopathological examination (Prof. Hultquist) The yellow tumour tissue consisted of large, swollen, cubic or polygonal cells. In some areas the cells were of the clear cell type heavily laden with lipids. The stroma was scanty but highly vascularized. The picture was characteristic of hypernephroma. In other areas, however the cells were arranged in an adenomatous pattern (fig 3—5)

The postoperative course was uneventful. The patient was transferred to the mental hospital. Up to now seven months later no deterioration of her state has occurred.

Discussion

It is well established that renal carcinoma has a definite predilection to metastasize to lungs, bones, lymph nodes,



Fig. 2) Left renal angiogram. The large swelling in the lower part of the kidney is while b) Right renal angiogram. In the upper part the hepatic artery is visualized and below the right renal artery and the renal swelling are seen.

liver and cerebrum. The tumours spread via the renal veins or via the lymphatics. A pyelorenal reflux may also mediate a tumour spread in instances of increased pressure of the urinary tract (10). There are numerous collaterals between the caval veins and the veins of the vertebral column, pelvis and retroperitoneal space thus giving many possibilities of metastatic spread. Direct metastasis from a renal neoplasm to the opposite kidney is uncommon. Metastatic ureteral involvement from a renal adenocarcinoma of the same or contralateral kidney (8, 12) or bilateral papillary carcinoma of the ureters or the pelvis is even rarer (5, 9). Bastable has made a review of twenty earlier cases of bilateral carcinoma of the kidneys (2). Several of these accepted cases may have been instances of metastases to the other kidney.

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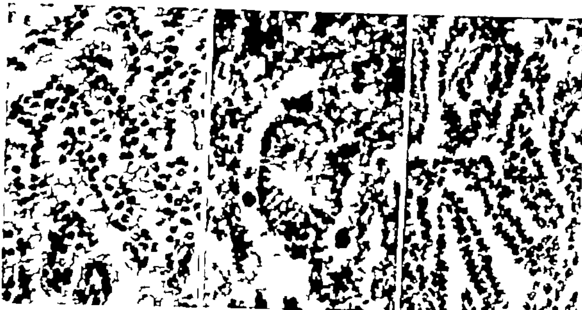


Fig. 3 Fig. 4 Fig. 5

Fig. 3—5 Photomicrographs of the tumour (Courtesy of Prof. G Hultquist.) Note the clear cell type of the cells in some areas heavily laden with lipids (Sudan black) In some areas there is an adenomatous pattern of the cells.

origin have been suggested for instance absence of other metastases or involvement of the renal veins long interval between the appearance of each renal tumour (2)

The author's case was diagnosed by laparotomy and biopsy from the left tumour The findings at the operation were in favour of primary bilateral involvement of the kidneys although metastases from one kidney to the other could not be excluded completely At the preoperative examination it was impossible to differentiate between polycystic kidney disease and malignant tumour The renal calcifications and the renal angiogram were perhaps in favour of malignancy There was however no distinct contrast filling of the tumours This finding and the absence of pulmonary metastases would be compatible with a low degree of malignancy It is possible that the tumours had undergone spontaneous regressive changes (1 6) Hultquist found a slight tendency towards a higher incidence of such changes among patients with tuberculosis and endocrine disturbances (6) The present case had small pulmonary calcifications but there were no signs of active tuberculosis. Nor was there any apparent evidence of endocrine disease.

Mental retardation in combination with renal tumours could be compatible with tuberous sclerosis (11) In 47 cases of tuberous sclerosis — all mentally retarded — nine patients came to autopsy and eight of them showed renal neoplasms. Seven of these showed numerous and sometimes innumerable tumours in both kidneys composed of various types of connective tissue (5) Another disease of the nervous system in combination with bilateral renal involvement is Lindau-von Hippel disease. Usually the renal component consists of cysts or benign tumours. The renal lesions may however be multiple bilateral primary hypernephro-

ma (7) There was no evidence of tuberous sclerosis in the present case. Nor were there any signs of retinal angiomata suggesting Lindau-von Hippel disease. Intracranial involvement of this disease would, however be impossible to exclude without cerebral angiography

The nomenclature of renal neoplasms is still confusing In the Anglo-American literature parenchymal tumours of the kidneys are called carcinoma or adenocarcinoma. According to Lubarsch solid tumours of alveolar structure are called hypernephroma. Such tumours are distinguished from malignant adenoma. According to that nomenclature the author case would be classified as hypernephroma. There were, however some features in the histopathological picture characteristic of malignant adenoma, as well. Many of the cases earlier reported are difficult to evaluate as to the classification. It is a general impression that many cases have been malignant adenomata and not hypernephromata.

Summary

A case of bilateral renal carcinoma is reported. The patient was mentally retarded. The abdominal masses did not cause the patient any apparent discomfort except some episodes of cystitis There were no evident signs of metastases outside the kidneys. The diagnosis was made by renal arteriography explorative laparotomy and biopsy The findings were in favour of primary bilateral involvement of the kidneys although metastases from one kidney to the other could not be completely excluded. Earlier cases of bilateral tumour involvement of the kidneys are mentioned, some of which were combined with disease of the nervous system.

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Haemodynamic Studies in Thyrotoxicosis

Before and after Treatment

By

A. M. Abrahamsen, J. Haarstad and C. Oulie

The haemodynamic changes in thyrotoxicosis are of particular importance. These changes have been the subject of many investigations (1–7).

Some authors have judged the circulatory conditions in thyrotoxicosis in relation to normal material (1–9) while others have investigated a series of patients with thyrotoxicosis before and after adequate treatment (8). One would, a priori, expect the latter method to give the most reliable results.

Humerfelt et al. (8) have previously reported on a series of patients with thyrotoxicosis from this department in which the circulatory changes were evaluated on the basis of the results of cardiac catheterization before and after treatment. This method will give very useful results, but it is doubtful whether one can obtain basal conditions under the stress of cardiac catheterization.

The dye dilution technique is very simple and puts no particular strain on the patient. One could expect to achieve almost uniform and basal conditions with this method of investigation, and it is therefore used in these haemodynamic studies.

Material

A total of 29 patients, 24 women and 5 men, were investigated before and after treatment. Their average age was 46 years. Three of the patients were admitted to hospital because of a relapse of thyrotoxicosis.

Concerning the treatment before the second examination, subtotal thyroidectomy was carried out on 17 patients. One of these was pretreated with I^{131}, one with Plummer's solution and Tapazole® (1-methyl-2-mercaptoimidazole), the remaining 15 with Plummer's solution only. Eleven were treated with Tapazole®, one in combination with I^{131}. One patient was given only I^{131}.

The average duration of symptoms was 16 months and varied from 2 months to 5 years. Enlargement of the thyroid gland was found in 23, and exophthalmus in 18 of the 29 patients.

On electrocardiographical investigation 23 patients showed sinus tachycardia, 4 auricular fibrillation, 1 paroxysmal auricular fibrillation and 1 atrial flutter. Three patients had moderate hypochromic anaemia, none had

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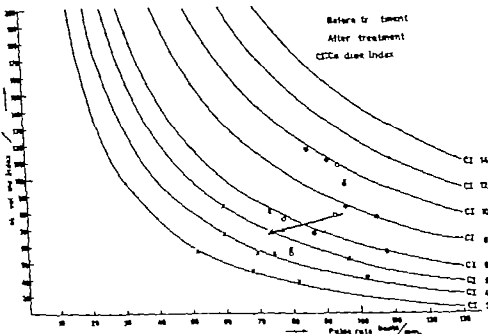


Fig. 1 The cardiac index before and after treatment.

Discussion

Many methods have been used previously in haemodynamic studies on thyrotoxicosis (1). The dye dilution technique has been little used (1). This method of investigation is harmless to the patients and there has been no difficulty in getting them to submit to further investigation after treatment. In a corresponding study in which cardiac catheterization was used (8) it was only possible to repeat the investigation in less than 50 % of the patients.

Three patients had moderate hypochromic anaemia, but this was not severe enough to influence the haemodynamic studies (2). These patients were given no treatment for this condition, and their anaemia remained unchanged throughout the course of the investigation.

On an average there is a slight fall in the stroke volume index after treatment. The arrow in fig. 1 marks the mean values before and after treatment. It will be seen that the cardiac index decreases markedly with fall in pulse rate, and also decreases somewhat with fall in stroke volume index.

In some patients with thyrotoxicosis the stroke volume is certainly increased. Anthonisen et al. (1) explain this on the basis of individual variations. Humerfelt et al. (6) found that in the patients with the highest cardiac output before their thyrotoxicosis was treated, a fall in stroke volume could be observed after treatment.

Fig. 2 shows that in our 29 patients there was a correlation between the size of the difference in cardiac index before

Table I Mean changes in observations

	Mean diff.	SE of mean diff.	
Basal metabolic rate (%)	− 42.8	3.43	$P < 0.001$
Plasma volume (l)	− 0.37	0.17	$0.025 < P < 0.05$
Blood volume (l)	− 0.72	0.27	$0.01 < P < 0.02$
Cardiac output (l/min)	− 3.7	0.68	$P < 0.001$
Cardiac index	− 2.4	0.43	$P < 0.001$
Stroke volume index (ml/m)	− 10.2	5.36	$0.05 < P < 0.10$
Pulse rate (beats/min)	− 21.8	2.14	$P < 0.001$
Appearance time (sec)	+ 3.3	0.50	$P < 0.001$

diastolic hypertension and one had diabetes mellitus. None of the patients had symptoms of heart failure when they were at rest.

The average heart volume of the 23 patients on X ray investigation was 405 ml/m² body surface, calculated according to Jonsell's formula.

Methods

Before starting treatment all the patients were investigated, fasting by the injection of 20 mg Evan's blue into the antecubital vein. The plasma volume and the blood volume were estimated. The dye dilution curve was recorded with a modification of Millikan's ear oxymeter with a technique described elsewhere (3 4)

The same investigations were repeated after the patients had completed their treatment.

Results

The results are seen in table I The basal metabolic rate fell in all patients following treatment. This drop varied from 11 % to 84 % averaging 42 8 % This reduction is significant ($P < 0\ 001$)

Both the plasma volume and the blood volume showed a tendency to fall the average fall being 0 37 l and 0 72 l, repectively The reduction in the blood volume is significant ($0\ 01 < P < 0\ 02$)

Both the cardiac output and the cardiac index were reduced after treatment, the mean reduction being 3 7 l/min. and 2.4 l/min /m² respectively ($P < 0\ 001$)

The stroke volume index showed an insignificant average drop (10.2 ml/m²)

The pulse rate (calculated from the dye dilution curves) dropped in 27 patients. The greatest fall was 49 beats/min in one patient the pulse rate remained unchanged while in another it increased by 2 beats/min. The pulse rate dropped on an average 21 8 beats/min. ($P < 0\ 001$)

The cardiac index was chiefly reduced by the fall in pulse rate. The stroke volume index remained almost constant, while in the patients with considerable drop in cardiac index the stroke volume index was also reduced In the patients in whom the cardiac index fell relatively little, the pulse rate dropped so much that there was a slight increase in the stroke volume index (fig 2)

There is a similar correlation between the differences in cardiac index and the differences in blood volume (fig 3)

The appearance time increased in all but one of the patients after treatment. The difference varied from 0 4 to 9 9 seconds with an average value of 3.3 seconds ($P < 0\ 001$)

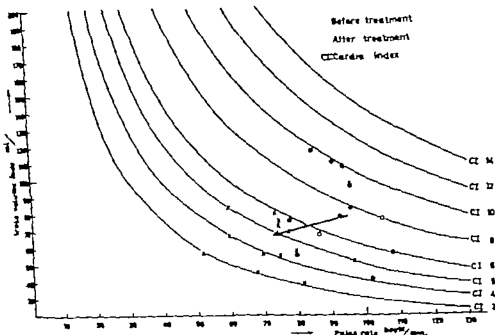


Fig. 1. The cardiac index before and after treatment.

Discussion

Many methods have been used previously in haemodynamic studies on thyrotoxicosis (1). The dye dilution technique has been little used (1). This method of investigation is harmless to the patients and there has been no difficulty in getting them to submit to further investigation after treatment. In a corresponding study in which cardiac catheterization was used (3) it was only possible to epeat the investigation in less than 50 % of the patients.

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Fig. 2 shows that in our 29 patients there was a correlation between the size of the difference in cardiac index before

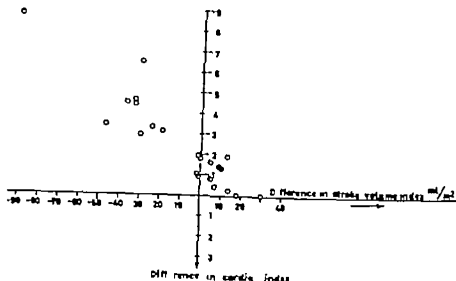


Fig 2. The correlation between differences in cardiac index and stroke volume index.

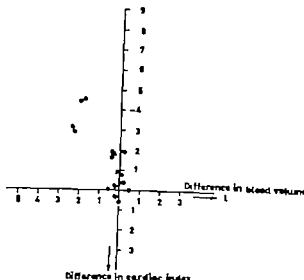


Fig 3 The correlation between differences in cardiac index and blood volume

and after treatment and the difference in stroke volume index. Great reductions in the cardiac index give a marked fall in stroke volume index, with smaller reductions the stroke volume index remains almost unchanged while with very small reductions, and in three cases with a very slight increase in the cardiac index, there was a slight increase in the stroke volume index.

It could be held, on the basis of the method of calculation, that this might be a spurious correlation An error in method could hardly give such a good correlation, but it might increase a correlation that was already present.

A significant fall in blood volume was found after treatment. This has also been found in other studies (5 6) while Anthonisen et al (1) found that there was no significant difference.

A similar curve to that in fig 2 applied to the changes in cardiac index and the changes in blood volume, will give a real, but not so marked correlation (fig 3) It is possible that the increase in

blood volume may be the cause of the increased stroke volume in some patients with thyrotoxicosis. The correlation between the difference in cardiac index and the difference in blood volume gives support to the opinion that the correlation between the difference in cardiac index and the difference in stroke volume is real.

As is to be expected and as remarked in earlier publications (1) the appearance time increases significantly after treatment.

The nervous lability in thyrotoxicosis may give considerable individual variations in the results, but using the dye dilution technique these variations probably will be as small as possible.

Summary

Haemodynamic studies, using the dye dilution technique, have been carried out on 29 patients with thyrotoxicosis, both before and after treatment.

After treatment there was found to be a significant fall in the cardiac index and the pulse rate. The mean values for the stroke volume index showed a slight fall. There was a clear correlation between the extent of the difference in cardiac index before and after treatment and the difference in stroke volume index.

With great reduction in the cardiac index there is a great drop in the stroke volume index, while with smaller reductions the stroke volume index remains almost unchanged. With small reductions, and in three cases with slight increase in the cardiac index, there is a slight increase in the stroke volume index.

The mean values for the blood volume decreased significantly following treatment, and there is a similar correlation between the differences in cardiac index and the differences in blood volume.

There was a significant increase in the mean values for the appearance time.

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Gamma-globulin in the Prevention of Viral Hepatitis

A Study on the Effect of Medium-size Doses

By

Trond Kluge

Up to the present, the most effective prophylaxis in viral hepatitis has been a high standard of general hygiene (9 19). Under conditions where satisfactory hygiene is difficult to maintain the question of inoculation prophylaxis deserves attention.

Our knowledge of hepatitis is still limited. Quite recently however American scientists succeeded in isolating a group of hepatitis viruses (6 20). This advance is probably an important step towards development of diagnostic antigens and vaccines.

At present, however serum immune-globulin or gamma-globulin (in the following referred to as G ") is our only method of inoculation prophylaxis.

The first successful trials with G in hepatitis were carried out by Stokes and Neefe (24), Havens and Paul (11) and Gellis et al (10). In the following years, series of investigations were carried out (13, 15 16, 25).

Most investigations have been carried out on epidemics of infectious hepatitis (IH) but recent trials seem to indicate a protective effect also against serum hepatitis (SH) (1).

G 's mode of action is not completely known, but most authors agree that a passive immunity against IH is obtained due to simple transfer of neutralizing antibodies. In addition an active immunity is superimposed on the passive one, in persons exposed to hepatitis virus (13 15 16 25).

Efficiency of gamma-globulin

Most authors agree that G gives some protection against viral hepatitis, but there is no agreement as to the degree of protection. As the titer of G must be high enough to prevent manifest disease, its effect depends on

a) Antibody content in the G preparation

G is prepared from large pools of human plasma, in which antibodies are supposed to be present. Their concentration is determined by the immune status

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of the donor group and will therefore vary from one pool to another

Direct measurement of hepatitis neutralizing antibodies cannot yet be performed but some information can be obtained from the plasma concentration and turnover of G The measurement is however complicated by the fact that G comprises a family of closely related proteins (17)

Studies on the distribution and turnover of injected G shows that the total body titer runs a bi phasic curve, with a rapid initial decrease (7) This is due partly to the mixing of G with extra vascular protein partly to degradation of G (21)

In 7—13 days an equilibrium is established between the intra and extra vascular pool of G and in the second phase the curve is linear G half lives calculated from this part of the curve vary from 21 to 26 average 23 days (7)

These results can most probably also be applied to the hepatitis antibodies. Consequently their persistence in the human body depends primarily on their initial titer

b) *The initial dose*

Administration of a small dose of 0 01 ml/lb body weight is reported to give a 90 % reduction of hepatitis incidence in children (13 25) About the same effect was obtained with as large doses as 0 06 ml/lb (11) and 0 15 ml/lb (24) Consequently a dose of 0 001—0 002 ml/lb seems to be sufficient in children

Studies on the necessary dose for adults are very few

Ward et al (26) found the small dose of 0 01 ml/lb body weight to give a 54 % protection in a population where 58 % were adults. In a group with adults only no protection was obtained

A study carried out by Gellis et al. (10) showed the large dose of 0 07 ml/lb body weight to give an average of 84 % protection among U S soldiers.

As hepatitis in adults usually is a more severe and prolonged disease than in children it seemed natural to suggest a larger optimum dose than for children. Susceptible adults with low natural immunity are recommended an inoculation of 0 06 ml/lb body weight before exposure to virus for a long period in an endemic area (5 16)

c) *The time of re inoculation*

The passive immunity occurs within 5 days (25) and lasts from 4 to 8 weeks (8, 25) During this period, an attenuated clinically inapparent infection will set up antibody production and result in active immunity which lasts from 4 to 6 months (15 24 25) Re inoculation will then not be necessary on the average 5 months have elapsed

If no such immunity develops, re-inoculation should be undertaken in 6—8 weeks.

Unfortunately we have no reliable methods to test the type and degree of immunity in inoculated individuals. The necessity of and time for re-inoculation must be determined from clinical experience and from the actual epidemiological situation.

The frequency of icteric and non icteric (an icteric) hepatitis — the I/A relation — is of special interest in the question of G prophylaxis

Quite different figures have been reported. The natural quotient (among non inoculated persons) has been estimated as 4 1 (4) 1 1 (11) 1 8 and 1 12 (15)

The effect of G on this relationship has not yet been determined.

The risk of anaphylactic reactions is considered very small (3). Transfer of serum hepatitis also is very unlikely (2, 3, 24). On the contrary G seems to protect against SH (1).

This paper is an attempt to answer the following questions:

1. The effect of G inoculation on military forces a) under highly endemic conditions b) in epidemics.

2. The optimum dose of G for adult persons.

3. The optimum time of re-inoculation.

Material and methods

In the Middle East with the Gaza area, viral hepatitis is very prevalent. The incidence among the civilian inhabitants during the last 6 years has been 2 % on the average, when only icteric cases are considered.

Since the United Nations Emergency Force (UNEF) was established in 1956, the prevention of hepatitis has been a salient problem (14).

The incidence has shown a mainly endemic pattern, with a maximum in December and January. Several epidemics have occurred, most of them among Scandinavian troops.

The present data have been collected over 6 years, covering the period November 1956—December 1962.

It is convenient to divide the UNEF troops into 3 main groups as follows:

a) *The Scandinavian units*, composed of officers between 25 and 50 years and soldiers between 20 and 30 years of age.

Records from the period 1950—1958 give an average hepatitis incidence of 0.06 % in the 3 Scandinavian countries. Their hygienic habits are very similar and the selection of personnel for UNEF service follows similar procedures. Consequently the above personnel represent a very homogeneous population with low natural immunity against viral hepatitis.

The Swedish and Danish-Norwegian (DANOR) battalions are situated close together in the Gaza strip and have almost identical ways of living, hygienic standards, medical services and relations with local civilians.

Table I. Viral hepatitis in Scandinavian units 1956—1962

Tenure 6 months

Unit	No. of men	No. of cases	% incidence
Swedish	6,296	28	0.45
Danish (DANOR)	7,150	47	0.66
Norwegian (DANOR)	5,492	39	0.71
UNEF Hospital (Norwegian)	1,829	28	1.53
Total	20,767	142	0.69

Table II. Viral hepatitis in the other units 1956—1961

Unit	No. of men	No. of cases	% incidence
Tenure 6 months			
Yugoslavian	6,949	4	0.05
Tenure 1 year			
Brazilian	3,037	28	0.92
Canadian	4,853	22	0.44
Indian	5,636	17	0.30
Total	13,526	67	0.50

Consequently their exposure to virus has been very uniform.

For the UNEF Hospital (Norwegian) the intimacy and duration of contact with patients implies a more intensive virus exposure.

The personnel is divided into 4 sections as presented in table I. The high incidence among personnel in UNEF Hospital is probably explained by the intensive exposure to virus.

The average incidence for all Scandinavian personnel is 0.69 %.

b) *The Yugoslavian unit*, which has by far the lowest incidence of viral hepatitis (table II), — this being explained by a high degree of natural immunity and by limited contact with local civilians.

Table III Viral hepatitis in Scandinavian contingents and its relation to G inoculations. No. of cases in

	Period					
	1956/57	1957	1957/58		1958	
	Contingent					
	I	II	III		IV	
	G			G	G	
Swedish	1/360	16/360	7/214	0/148	1/512	
Danish.	1/550	1/550	2/550		10/550	
Norwegian	0/337	1/295	0/428		14/417	
						G
Hospital	0/207	1/223	5/124		9/121	0/121

G = γ-globulin given.
5/124 signifies 5 cases per 124 soldiers.

c) *The Brazilian, Canadian and Indian units* (table II) have an average incidence of 0.50 %. This high figure is somewhat surprising as viral hepatitis is quite prevalent both in Brazil and India.

Only icteric cases have been recorded. All patients have presented a typical story and picture of viral hepatitis. The majority have been examined in UNEF Hospital, with ordinary laboratory tests (Serum Colour ESR, Harrison s and Schlesinger's reactions)

The IH/SH relation cannot be accurately estimated but most cases have been infectious hepatitis (IH) It should be noted, however that UNEF personnel receive numerous injections before and during their service (14)

Due to very limited laboratory facilities, liver function tests have been performed only in a few cases. Hence no estimate can be made of the frequency of non-icteric hepatitis.

All cases are obliged to be reported to UNEF Medical Section. The system has been reliable, and almost every case of icteric hepatitis has been recorded.

Only Scandinavian troops have received G Due to different views on the value of G., the use, dosage and re inoculation have differed between the 4 national groups, and from one period to another

The G preparations have been Austrian (Institut Autrichien d'Hemoderivés) and Swedish (A/B Kabi)

As far as we know no acute complications have occurred Nor is there any suggestion that G inoculations have induced serum hepatitis.

Table III gives a survey of viral hepatitis in the 4 Scandinavian groups — and its relation to G inoculations. The hepatitis incidence is considerably lower in contingents subject to G prophylaxis.

Results

1 *The protective effect of G in Scandinavian UNEF troops*

The estimations are based on the incidence (attack rate) of icteric hepatitis among inoculated and non-inoculated personnel as presented in table IV The hepatitis incidence among inoculated Scandinavians is only 1/10 of the incidence

...lation to G prophylaxis

Period								
1958/59	1959	1959/60	1960	1960/61	1961	1961/62	1962	1962/63
Contingent								
V	VI	VII	VIII	IX	X	XI	XII	XIII
G 0/513	G 1/639	G 0/660	G 1/680	G 1/442	G 0/442	G 0/442	G 0/442	G 0/442
2/330	1/550	14/550	3/330	7/330	3/550	G 1/550	0 550	0/550
G 0/415	G 1/474	19/479	0/485	3/478	1/484	G 0 485	G 0/484	G 0/483
G 0/121	G 0/121	G 0/121	10/121 G 0/121	G 3/121	G 0/121	G 0/122	G 0/122	G 0/122

Table IV Attack rate of viral hepatitis. Degree of protection from γ-globulin

Unit	Not inoculated			Inoculated			% protection
	No. of men	No. of cases	% incidence	No. of men	No. of cases	% incidence	
Swedish	574	23	4.00	5,722	5	0.09	97.8
Danish (DANOR)	6,600	46	0.70	530	1	0.18	74.3
Norwegian (DANOR)	3,403	38	1.11	2,343	1	0.04	96.3
Total DANOR	10,003	84	0.84	2,893	2	0.07	91.7
UNEF Hosp.	796	25	3.14	1,213	3	0.25	92.0
All Scandinavians	11,373	132	1.14	9,828	10	0.10	91.3
Brazilian Indian/Canadian	13,326	67	0.50	–	–	–	–
Yugoslavian	6,949	4	0.03	–	–	–	–

in the Scandinavian control group, and only 1/5 of the incidence in the control group composed of Brazilian, Indian and Canadian soldiers.

The average degree of protection among the 9 828 inoculated persons is 91.3 % a result which must be considered very satisfactory

Table V Epidemics of viral hepatitis Effect of γ-globulin

Contingent	Period	Not inoculated		Inoculated		% protection
		No. of men	No. of cases	No. of men	No. of cases	
Swedish II	Apr /Oct. 57	360	16	–	–	–
Swedish III	Oct. 57—Apr 58	214	7	148	0	100
DANOR IV	Apr /Oct. 58	967	24	–	–	–
DANOR V	Oct. 58—Apr 59	550	2	415	0	100
UNEF Hosp. IV	Apr /Aug. 58	121	9	–	–	–
	Aug./Oct. 58	–	–	121	0	100
UNEF Hosp. VIII	Apr /Sept. 60	121	10	–	–	–
	Sept./Nov 60	–	–	121	0	100

Table VI. Hepatitis incidence of inoculated personnel classified according to dosage and time of re-inoculation

Unit	No. of men	Dose (ml/kg)	Time of re inoculation	No. of cases	% incidence	Interval between inocul. and dis.
Norw (DANOR) + UNEF Hosp. (Norw)	1 373	0.014	3 months	1	0.07	4 months
Swedish	1 168	0.040	–	1	0 09	6 months
Swedish	3,529	0.040	0.028 3 months	3	0 09	4 months (1) 10 months (2)
Swedish	1 025	0.040	6 weeks	1	0.10	10 months
Norw (DANOR) + UNEF Hosp. (Norw.)	1,820	0 043	3 months	0	0	–
UNEF Hosp. (Norw)	363	0.047	4 weeks	3	0.83	4 months (2) 5 months (1)
Danish (DANOR)	550	0.147	–	1	0 18	Unknown

2 *Effect on epidemics*

The effect of G inoculations in epidemics is presented in table V The epidemic in the Swedish contingents II and III did not affect the inoculated personnel Two epidemics in UNEF Hospital vanished after inoculation of all personnel

3 *Dosage of gamma-globulin*

The inoculated contingent of Danish personnel received one single injection of 0 147 ml/kg and no re-inoculation. Thus, their G administration differs very much from the other groups. It is seen (table VI) that their protection is no greater than that obtained with the mini

mum dose of 0.014 ml/kg. The Danish group however is too small to permit safe conclusions.

80 % have received an average dose of 0.040 to 0.047 ml/kg body weight. Their rate of protection, varying from 92 to 98 % (table IV) indicates this to be an adequate dose for adult persons in hepatitis endemic areas. The few cases of hepatitis have occurred from 4 to 10 months after inoculation.

4 *Time of re-inoculation*

It is seen from table VI that the time intervals have varied from 4 weeks to 5 months. Three Swedish and 1 Danish contingent have not been re-inoculated.

Altogether 10 cases of hepatitis have been recorded in the inoculated group of personnel. The highest incidence is notified for UNEF Hospital, contingent IX. Two of the 3 cases were recapitulants not subject to regular re-inoculations. Their disease appeared respectively 4 and 5 months after last inoculation.

Discussion

1 The present study comprises the largest number of G inoculated individuals that has hitherto been published.

Except for the UNEF Hospital, the material of Scandinavian soldiers must be considered very homogeneous. The *high average rate of protection* (91 5) indicates C to be a very valuable prophylaxis against viral hepatitis.

N information is btained on the non-icteric hepatitis in UNEF. This is deplorable, but on the other hand the material is probably more exact, as the non-icteric hepatitis is very easily confused with other conditions.

The effect of G on the I/A relation (vide supra) can be as follows

a) No effect either *on the totals of I and* A cases, or on their relative frequency Provided that a sufficient dose of G is given, this possibility is small.

b) Reduced number of I unaltered number of A cases.

c) Both I and A reduced.

d) Reduction of I increase of A — possibly an unaltered total number. The effect of G. will then be a transformation of icteric to non-icteric cases.

A study on 157 patients by Drake and Ming (8) suggested the presence of the conditions in b) or d) above, depending on the dose of G

Krugman and Ward (15) in a study on 45 *patients, found* a relation as in d) above.

The I/A-relation is probably influenced by several factors, such as the immune status of the patients and the amount and virulence of virus. Besides, the combination of passive and active immunity represents degrees *of protection varying* with the dosage of G., and operating over a wide range of time intervals between inoculation and virus exposure.

The only conclusion to be drawn, is that further investigations of the I/A relation before and after G inoculation should be undertaken.

So should studies on the frequency of re-infections (which are considered rare) and of late complications, mainly liver cirrhosis.

With our present knowledge, however it should be tated that G represents a very valuable prophylaxis against viral hepatitis.

2 A good effect is obtained in the control of epidemics. On the other hand, the *epidemic in the Danish* contingent IV and Danish-Norwegian contingent VII decreased without G inoculations. This may be explained by

a) A more strict adherence to hygienic precautions when an epidemic occurs.

b) A change in the epidemiological situation of the area — perhaps among the local civilians.

c) G inoculation of one part of a battalion will contribute to the protection of the other part e.g the DANOR battalion V

The hepatitis incidence is dependent on the duration and intimacy of virus exposure (16) This is clearly demonstrated by the high incidence and many epidemics among UNEF Hospital personnel, whose contact with hepatitis patients has been intimate.

3 Under the present circumstances the Yugoslavian unit does not seem to need G prophylaxis. For the rest of the UNEF troops, the investigations indicate that G prophylaxis should be undertaken

4 The doses of G have been adequate, and an average dose of 0 04 ml/kg seems to give a satisfactory protection This is only 1/3 of the dose recommended by previous authors.

As great expense is involved in the use of G a reduction of the necessary amount is of economic importance.

5 The present study does not give any accurate estimation of the optimum time of re-inoculation as only 10 cases are recorded among inoculated personnel It seems that an interval of 5 months should not be exceeded

Summary

The incidence of viral hepatitis and its relation to gamma-globulin prophylaxis has been investigated in a total of 21 201 Scandinavian soldiers serving in the United Nations Emergency Force in the Middle East.

The material is considered homogeneous, with a similar low natural immunity and a similar exposure to hepatitis virus during the service in the Middle East.

Only icteric cases are recorded, most of them being of the infectious type ("type A hepatitis)

1 In the inoculated group of 9,828 soldiers the hepatitis incidence was on the average 0 10 per cent.

In the non-inoculated group of 11,373 soldiers, the incidence was on the average 1 14 per cent.

This gives an overall protection rate of 91.3 per cent.

In a second control group consisting of 13 526 Brazilian Canadian and Indian soldiers who did not receive gamma globulin, the incidence was on the average 0 50 per cent.

The investigations suggest that gamma-globulin be recommended for 6 of the 7 national contingents serving in UNEF

2 A good protective effect seems to be obtained in epidemics. The value of G prophylaxis is the greatest when general hygienic precautions are difficult to maintain.

3 The investigations indicate that a dose of about 0 04 ml/kg (0 02 ml/lb) body weight gives a satisfactory protection of adult persons.

This is only one-third of the dosage which hitherto has been recommended

4 Immunity seems to last about 5 months. This observation corresponds well with results from previous investigations.

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Electrocardiographic Findings at Rest, during and after Exercise in Healthy Old Men Compared with Young Men

By

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Changes in the scalar resting electrocardiogram of adults with rising age have been quite extensively studied (29 53) With rising age changes take place, *inter alia*, reductions in the sum of the QRS and T-wave amplitudes in the standard leads in conjunction with a reduction in amplitude of the mean spatial QRS and T vectors. At the same time the mean QRS vector shifts to the left in the frontal plane whereas the change of the T vector is less distinct. The P—R interval becomes longer at a high age, while the QRS-interval, at least in some studies, has remained unchanged up to 60 years of age.

When normal values for young persons are used as criterion for assessment of the electrocardiogram, abnormal findings have been reported in between 30 and 85 per cent of clinically healthy persons bove 70 years of age (12, 29 53) The most common findings vary in different studies, comprising ectopic beats, A—V block I low QRS voltage, Q-wave changes, intraventricular conduction disturbances, depression of the ST segment, and flattening or inversion of the T wave.

The data are more scanty concerning the electrocardiographic changes in conjunction with physical exertion at different ages. But a higher frequency of especially ST depressions and sometimes, too, of T-wave depressions have been recorded with rising age after the Master two-step test (31 40 51) after submaximal treadmill work for 15 minutes (52) strenuous bicycle exercise for brief periods (6) and running up and down stairs at maximal speed for 1—1 1/2 minutes (1 48)

There are few comparisons, however of the electrocardiographic findings both during and after exercises at different ages (2 3 4 6 7 13 16) Within the 40—64 age group the incidence of abnormal electrocardiographic changes in an exercise test, mainly ST—T depressions and ectopic beats, has been reported as between 8 and 37 per cent (2, 3 6 15 16, 31 48) Above 60 years of age no major series of exercise electro-

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Table I. Some anthropometric and clinical data in the different age groups. Means, S. D. and S. E. of means are given

Age group (yrs)	Source	Rejected: No. of individ.		Accepted: No. of individ.		Age (yrs)	Height (cm)	Weight (kg)	B. P. Systolic	B. P. Diastolic	Physical training Earlier 1	Earlier 2	Earlier 3	At examin. 1	At examin. 2	At examin. 3
30–39			3		48	33.8	176.9	72.5	127.2	79.1	25	18	5	28	17	3
							6.8	7.3	9.3	6.8						
							1.0	1.1	1.3	1.0						
40–49			5		24	44.9	173.2	75.2	131.4	81.8	4	13	3	11	8	1
							5.8	9.1	9.6	6.3						
							1.2	1.9	1.9	1.3						
50–59			7		15	55.1	174.7	73.1	134.5	85.5	2	7	5	6	5	3
							7.8	8.5	9.7	7.7						
							2.0	2.3	2.5	2.0						
60–69		6		12			173.9	74.2	146.4	87.1	4	10	7	4	15	2
	b	6		7			6.4	8.7	11.4	5.9						
		3	17	2	22	63.0	1.4	2.1	2.4	1.3						
	d	1		1			—	—	—	—						
		1					—	—	—	—						
70–79		4		2			172.7	73.5	148.6	82.3	0	7	3	3	6	1
	b			1			3.7	8.7	9.8	6.1						
		4	17	4	15	72.5	1.0	2.4	2.9	1.8						
	d	3					—	—	—	—						
		6		6			—	—	—	—						
80–83							175.5	72.8	150.0	72.5	0	2	2	1	3	0
	b			1			8.5	15.3	14.1	9.6						
			1	1	4	81.0	4.5	7.7	7.0	4.8						
	d	1		1			—	—	—	—						
				1			—	—	—	—						

= Subjects invited from the Health Survey of the City of Stockholm.
b = Subjects invited from the Labour Exchange.
= Subjects invited from an old age home, mainly upper and middle class, ~ 100 men informed.
d = Subjects invited from old age homes, mainly lower and middle class, ~ 300 men informed.
= Subjects invited from gymnastic groups for aged, ~ 30 men informed.

1 = No regular physical training.
2 = Moderate degree of training.
3 = High degree of training such as hard bicycling every day, cross-country running or hard work, g in the building trade.

Anamnestic data of physical activities have not been given by 11 individuals.

supine position 1/2—1 min. after and 4 min. after exercise. If changes in the ECG persisted, recording was also made 10 min. after exercise. The paper speed used was 50 mm/sec. and each recording of the four-lead ECG was obtained in approximately 10 sec. At rest leads, I, II, III VR, VL, aVF $CR_{(apex)}$ and, in the later part of the

cardiograms of healthy individuals has been published

The present investigation was performed in order to study the frequency and type of electrocardiographic findings at rest and during and after a standardized exercise test in a group of healthy old males and to compare with the findings in younger age groups.

Material

All the subjects up to 59 years of age, and some of those above that age were drawn from the 1954 Health Survey of the City of Stockholm (15 16) The latter survey comprised subjects randomly selected from the City of Stockholm of the men invited 68 (1 097 subjects) appeared for examination. For the present investigation, subjects in whom disease was not diagnosed in the Health Survey were randomly selected and invited to take part in a new health investigation. Invitations were also extended to about 30 further subjects from the Health Survey aged 64—73 years, who had had no essential diagnosis. Diagnoses accepted as non-essential were, for example slight spondylosis deformans, moderate varicose veins and haemorrhoids, inguinal hernia, and moderate overweight. Sixty nine % of the subjects accepted the invitation, and the study of these men started during the autumn of 1958. Further details concerning the selection of this part of the material have been published earlier (9)

In the ages above 60 years the material was further increased by announcing the investigation in homes for the aged in Stockholm. The announcements stated that healthy males aged 60 or above could obtain a free medical examination including a thorough study of the heart and lung functions. Announcements were also posted at the Stockholm labour exchange. Subjects taking part in organized gymnastics for old people in Stockholm were also invited. Those who were or had been obviously diseased were rejected before investigation. The numbers of subjects from different sources are given in table I The selection of the material above 60 years of age will be discussed below

EXAMINATION OF THE MATERIAL

The material to be examined comprised 176 men between 30 and 83 years. All stated that they were in good health and had had no recent infection. The clinical examination consisted of a complete case history physical examination, blood tests (ESR according to Westergren in most subjects, haemoglobin concentration) urinary examination for protein and reducing substances, and measurements of weight and height.

REJECTION

Table I shows the number of subjects in the different age groups who were rejected as not clinically healthy

Case history Subjects reporting a history suggestive of angina pectoris /8/ intermittent claudication /2/ or chronic bronchitis /2/ were excluded, as well as subjects who had had suspected rheumatic fever /8/ pleurisy /5/ pulmonary tuberculosis /3/ or other major disease /4/

Physical examination. Subjects with elevated blood pressure at rest /12/ as judged by the upper normal limit (e. g 160/100 or 175/80 mm Hg above 60 years of age) given by Master et al. (36 37) were not accepted, nor subjects with frequent ectopic beats in bigeminy or trigeminy /2/ or atrial fibrillation /1/ One subject was rejected because of gross overweight (height 168 cm, weight 95 kg).

Laboratory tests. Below the age of 50 no subject was rejected because of high ESR, the highest value observed being 13 mm/hour Above the age of 50 the highest value accepted was 15 mm/hour one subject was rejected because of an ESR of 53 mm/hour One case was rejected because of proteinuria. There were no rejections due to abnormal haemoglobin values. The lowest value observed for subjects below 50 years of age was 12.2 g/100 ml, and for those above 50 years 11 4 g/100 ml.

Methods

Electrocardiogram

ECG was recorded with a direct writing apparatus (Mingograf 42, Elema Järnh., Stockholm) at rest in the supine and standing positions, during exercise on a bicycle ergometer in the sitting position, and in the

electrocardiographic recording after 2, 4 and 6 min. at each load and before the interruption of the test. In most of the subjects below the age of 60 the test was interrupted before exhaustion if a heart rate of or close to 170 beats/min. was reached (58). Above the age of 60 years this was true for the first few subjects examined, thereafter the intention was to obtain the highest possible heart rates at these ages. In 31 subjects of 60 years of age or above the exercise test was repeated t least once in the sitting and/or supine position.

Statistical calculations. Current statistical methods were used (54) unless otherwise stated.

Results

Heart rate at rest, final heart rate during exercise and final load in the different age groups are given in table II. The average pulse rates at rest are essentially the same in the different age groups with the exception of the youngest and the oldest, whereas the final heart rate and final load diminish successively with rising age. The cases with abnormal ECG (see below) did not differ significantly from the remainder in respect of anamnestic degree of physical activity final heart rate or final load. During the exercise test only one of the subjects with abnormal ST depressions showed angina pectoris (73 years of age)

The incidence and classification of electrocardiographic changes at est during the orthostatic and exercise tests in different age groups are presented in tables III and IV and fig. 1 In two cases of bundle-branch block ST—T changes at rest and during exercise have not been recorded in table III nor ST—T changes during exercise in one case of marked ST—T depressions in the resting ECG indicative of myocardial injury These three cases have been excluded from table IV and fig 1

AT REST IN SUPINE POSITION

All five individuals who had ectopic beats were 60 years of age or above.

Left axis deviation of —30°— —90° was fairly common in the higher age groups. In 9 of the 10 subjects with this finding the ECG was consistent with peri-infarction block (19) with a wide angle between the initial and the ter minal QRS vector in the frontal plane. All these subjects had normal ST segments at rest. The positions of the mean electrical axis in the two cases of bundle branch block are not listed in table III.

ST depressions classified as abnormal were recorded in only one subject (66 years old) who also showed negative T waves as in old myocardial injury In only one of the 12 cases with flattening of the T wave was there simultaneous ST depression

AT REST IN STANDING POSITION

Orthostatic electrocardiographic changes were recorded in 14 % of the cases in the 30—39 and in only 2.5 % in the 40—83 age group None of these subjects showed horizontal or sagging ST depressions in the exercise test.

DURING EXERCISE TEST

Ectopic beats Occasional supraventricular ectopic beats were recorded in all age groups except the youngest, but frequent supraventricular ectopic beats were recorded only in the 70—83 age groups. Ventricular ectopic beats were recorded in all age groups, although more frequent in the highest decades. In the subjects below 60 years of age there were five cases of ventricular ectopic beats from the left ventricle (QRS pattern as in right bundle-branch

Table II Heart rate at rest in supine and standing position and final heart rate and work load during exercise in sitting position in the different age groups. Means, S D and S E. of means are given

Age group (yrs)	No. of Individ.	At rest		During exercise	
		Heart rate (beats/min)		Final heart rate	Final work load
		Supine	Standing	(beats/min)	(kpm/min)
30–39	48	67.5	85.6	173.8	1 004
		10.8	13.9	8.5	174
		1.6	2.0	1.2	25
40–49	24	67.3	80.2	172.0	1 004
		12.2	13.5	11.1	168
		2.5	2.8	2.3	34
50–59	15	65.3	80.3	163.5	942
		10.2	14.9	15.7	188
		2.6	3.8	4.0	49
60–69	22	68.4	81.1	154.3	815
		8.4	12.3	17.4	175
		1.8	2.6	3.7	37
70–79	13	67.5	80.7	153.3	740
		8.7	12.9	14.7	155
		2.4	3.6	4.1	43
80–83	4	61.3	71.5	134.0	631
		13.6	13.9	13.8	75
		6.8	7.0	9	37

study also $V_{1-3, 5, 7}$ were used in the standing position only I II III $CR_{1, 4, 5}$, were recorded. During exercise ECG was recorded after 5 min. at each load. For heart rate determination a recording was also mad after 2 4 and 6 min. at each load of generally at least 20—30 beats (paper speed 25 or 10 mm/sec.) in some cases 10—15 beats (25 mm/sec.) During exercise the indifferent electrode was placed on the forehead (57 58) and $CH_{2, 4, 5, 7}$ were recorded. CH-leads have been shown to be approximately identical to CR-leads except in cases with markedly abnormal ST and T vectors (25)

The electrocardiographic changes at rest, during and after exercise, were classified according to published criteria (20 53) and the experience of this laboratory In table III the rules are given for the classification o the changes observed. Such brief rules, however cannot cover all aspects of the evaluation of electrocardiographic findings. Many other factors may have to be considered when judging the significance of changes, e g. in ST and T in the individual case. The pattern of the changes in the different leads, the appearance of new changes, the temporal evolution of the changes during an exercise test, and comparison with changes during the orthostatic test, are some of these factors. In only a few cases have these factors affected the classification in the present investigation. ST—T changes which seemed to be associated with the increase in heart rate during exercise (55 56) were generally of the type classified as slightly different from normal (table *III*). As a rule the ST depressions have been measured from a horizontal line through the end of the preceeding P—R segment. When the P—R segments have sloped, e. g. because of a positive afterpotential or a T wave, this has been taken into consideration (31) In all age groups the electrocardiographic findings have been classified according to the same rules, and the classification has been repeatedly checked by comparison between records with similar changes.

Orthostatic test

Heart rate and ECG were recorded after 8 min. standing with the back of the head leaning against a wall. The classification of the electrocardiographic changes is given in table III (23)

Exercise test

The subjects were exercised in the sitting position on an electrodynamically braked bicycle ergometer (24) starting at a load of 300 kpm/min. This was increased stepwise by a further 300 kpm/min. until the subject was exhausted or the ECG contraindicated further exercise. Marked horizontal ST depressions or an increasing number of ectopic beats at high relative loads were regarded as relativ contraindications. In a few cases the chosen loads differed The final load was taken to be the last load at which the subject worked for 6 min., with a proportional increment if he completed part of the period at the next higher load. Heart rate was determined by

Table III. (cont.)

	Classification	Incidence of changes						
		Years						
		30–39	40–49	50–59	60–69	70–79	80–85	30–85
		No. of subjects						
		48	24	14	21	12	4	123
ECG changes during an exercise test								
Rhythm								
Occasional supraventricular ectopic beat( ) during or after exercise	II	—	2	3	5	2	2	14
Moderately frequent supraventricular ectopic beats during or after exercise	III	—	—	—	—	1	—	1
Frequent supraventricular ectopic beats, two or more in series during or after exercise	V	—	—	—	—	4	1	5
Occasional ventricular ectopic beat( ) during exercise t low relative loads or after exercise	III	1	—	1	—	—	—	2
Occasional ventricular ectopic beat(s) during exercise at high relative loads or moderately frequent ventricular ectopic beats during exercise t low relative loads or after exercise	IV	2	3	—	3	1	—	9
Increasing number of ventricular ectopic beats during exercise, frequent, two or more in series or from more than one focus, during or after exercise	V	—	1	2	3	3	1	10
AV-block I fter work (PQ = 0.23 sec)	III	—	—	—	1	—	—	1
ST T[1]								
ST-junction depression < 0.5 mm	II	12	3	3	7	3	—	30
ST-junction depression ≥ 0.5 but < 1.5 mm or change to horizontal ST without depression 4 min after exercise	III	3	5	1	4	2	1	18
Horizontal or sagging ST-segment depression 0.5 mm, or ST-junction depression _ 1.5 mm	IV	2	—	3	3	1	1	10
Horizontal or sagging ST-segment depression _ 0.5 mm generally most marked 4 min after exercise	V	—	1	—	—	4	—	5
Moderate flattening of the T wave during or after exercise	II	7	2	—	3	3	—	15
Marked flattening of the T wav during or after exercise	III	3	3	1	—	1	—	8
Isoelectric T wave after exercise	IV	—	—	—	—	1	—	1

I Normal ECG, II = Slightly different from normal, III = Intermediate, IV = Suspected abnormal, V Abnormal, VI = Moderate orthostatic changes, VII = Marked orthostatic changes.

Changes in ST T refer to leads I, II $CR_{4,5,6}$, $CH_{4,5,6}$ and in some cases III, VL, VF

Three subjects with abnormal changes in QRS or ST T t rest are excluded, see text.

Table III ECG changes observed at rest, during an orthostatic test and during an exercise test in the different age groups. Some subjects showed more than one type of change the most marked change being listed for each type

	Classification	Incidence of changes						
		Years						
		30–39	40–49	50–59	60–69	70–79	80–83	30–83
		No. of subjects						
		48	24	15	22	13	4	126
ECG changes at rest								
Rhythm								
Heart rate ≥ 95/min (sinus tachycardia)	II	[illegible]	1	—	—	—	—	5
Heart rate ≤ 50/min (sinus bradycardia)	II	—	2	1	—	1	1	5
Occasional supraventricular ectopic beat(s)	II	—	—	1	—	—	—	1
Frequent supraventricular ectopic beats, two and more in a series	V	—	—	—	—	1	—	1
Occasional ventricular ectopic beat(s)	III	—	—	—	2	—	—	2
Ventricular ectopic beats from two foci	V	—	—	—	—	—	1	1
Ectopic auricular rhythm	III	1	—	—	—	—	—	1
QRS								
Right axis deviation, 90—110	II	—	—	2	—	—	—	2
Extreme left axis deviation, -30—-90	IV	1	—	1	4	1	3	10
Left axis deviation, -1—-29°	II	1	2	4	3	2	—	12
Incomplete right bundle branch block	II	2	—	—	—	—	—	2
Incomplete left bundle branch block	II III	2	—	—	2	—	—	4
Right bundle branch block	V	—	—	—	—	1	—	1
Left bundle branch block	V	—	—	1	—	—	—	1
Q wave not entirely normal	II III	—	1	—	1	—	—	2
ST-T								
ST-junction depression < 0.5 mm, or near horizontal ST-segment without depression	II	1	1	1	—	2	—	5
ST-junction depression 0.5—1 mm, or horizontal ST-segment without depression	III	1	3	—	1	1	—	6
Horizontal ST-segment depression < 0.5 mm	IV	—	—	—	—	1	—	1
Horizontal ST-segment depression 0.5—1 mm	V	—	—	—	1	—	—	1
Slightly flattened T wave	II	3	—	2	3	—	1	9
Moderately flattened T wave	III	—	—	—	1	2	—	3
Negative T wave	V	—	—	—	1	—	—	1
ECG changes during an orthostatic test								
Isoelectric or slight diphasic T in II III CR4 or CR7	VI	6	—	1	—	1	—	8
ST-depression < 1 mm and flattened T in II, III, CR5–7	VII	1	—	—	—	—	—	1

(I, II III, IV V see table III)

Age groups (yrs)											
60—69 (21 subjects)				70—79 (12 subjects)				80—83 (4 subjects)			
Total	EB	QRS	ST T	Total	EB	QRS	ST T	Total	EB	QRS	ST T
8 (38)	19 (90)	12 (57)	16 (76)	5 (42)	11 (92)	9 (75)	7 (58)	—	3 (75)	1 (25)	3 (75)
6 (29)	—	4 (19)	3 (14)	3 (25)	—	2 (17)	2 (17)	1 (25)	—	—	1 (25)
3 (14)	2 (10)	1 (5)	2 (10)	2 (17)	—	—	2 (17)	—	—	—	—
4 (19)	—	4 (19)	—	1 (8)	—	1 (8)	1 (8)	2 (50)	—	3 (75)	—
—	—	—	—	1 (8)	1 (8)	—	—	1 (25)	1 (25)	—	—
—	11 (55)	—	5 (24)	1 (8)	4 (34)	—	2 (17)	—	1 (25)	—	2 (50)
9 (43)	4 (19)	—	9 (43)	2 (17)	1 (8)	—	3 (25)	—	1 (25)	—	—
4 (19)	—	—	4 (19)	—	—	—	2 (17)	1 (25)	—	—	1 (25)
4 (19)	3 (14)	—	3 (14)	1 (8)	1 (8)	—	1 (8)	1 (25)	—	—	1 (25)
4 (19)	3 (14)	—	—	8 (67)	6 (50)	—	4 (33)	2 (50)	2 (50)	—	—
—				1 (8)				—			
5 (24)				2 (17)				—			
5 (24)				—				1 (25)			
7 (33)				1 (8)				1 (25)			
4 (19)				8 (67)				2 (50)			

The figures in brackets give the frequency in %.

after exercise was recorded in only one case. Most subjects with flattening of the T wave in the exercise test showed a simultaneous depression of ST while most ST depressions in the exercise test were not combined with flattening of the T wave (table V). In no case was a negative T wave observed during or after exercise except in one with bundle-branch block at rest. In the man with ST depressions and T wave negativity in the resting ECG indicative of myocardial injury the ST depression diminished during and immediately after exercise and the T wave became positive.

Correlation between ectopic beats and ST depressions. The lack of correlation between ectopic beats and ST depressions during and after exercise is shown in table VI. Only in the 50—59 age group was there in cases with ventricular ectopic beats a significantly higher incidence ($p < 0.01$) of abnormal or suspected abnormal ST depressions compared with

Table IV The incidence of ECG changes recorded at rest and during an exercise test, classified in fo

	Age groups (yrs)											
	30–39 (48 subjects)				40–49 (24 subjects)				50–59 (14 subjects)			
	Total	EB	QRS	ST T	Total	EB	QRS	ST T	Total	EB	QRS	S
ECG at rest												
I	35 (73)	47 (98)	42 (87)	43 (90)	15 (63)	24 (100)	21 (88)	20 (83)	5 (36)	13 (93)	7 (50)	1
II	10 (21)	—	5 (11)	4 (8)	6 (25)	—	2 (8)	1 (4)	8 (57)	1 (7)	6 (43)	5
III	2 (4)	1 (2)	—	1 (2)	3 (12)	—	1 (4)	3 (13)	—	—	—	—
IV	1 (2)	—	1 (2)	—	—	—	—	—	1 (7)	—	1 (7)	—
V	—	—	—	—	—	—	—	—	—	—	—	—
ECG during exercise test												
I	26 (54)	45 (94)	—	26 (54)	11 (46)	19 (79)	—	13 (54)	6 (43)	10 (72)	—	7(
II	13 (27)	—	—	15 (31)	5 (21)	1 (4)	—	5 (21)	3 (22)	1 (7)	—	3(
III	6 (13)	1 (2)	—	5 (11)	3 (13)	—	—	5 (21)	2 (14)	1 (7)	—	1
IV	2 (4)	2 (4)	—	2 (4)	3 (12)	3 (13)	—	—	1 (7)	—	—	3(
V	1 (2)	—	—	—	2 (8)	1 (4)	—	1 (4)	2 (14)	2 (14)	—	
ECG at rest and during exercise test												
I		19 (40)				9 (38)				2 (14)		
II		17 (36)				6 (25)				6 (43)		
III		8 (16)				4 (17)				2 (14)		
IV		3 (6)				3 (12)				1 (7)		
V		1 (2)				2 (8)				3 (22)		

Total = All changes. EB = Ectopic beats. QRS = Changes in QRS. ST T = Changes in ST T

block) four of ventricular ectopic beats from the right ventricle (QRS pattern as in left bundle branch block,) and four of ventricular ectopic beats from ventrally localized foci in which it could not be decided from which ventricle the ectopic beat derived. In the subjects of 60 years or above ventricular ectopic beats were recorded from the left ventricle in four cases, from the right ventricle in eight, from ventral foci in four and from dorsal foci in two

ST—T changes The incidence of ST depressions during and, especially after exercise increased with age. No significant relationship between the degree of ST depression and the blood pressure at rest was found in the different age groups, nor between the assessment of ST and T at rest and of ST during and after exercise. Slight to moderate flattening of the T wave during and after exercise was less common than depression of the ST segment isoelectric T wave

Table VI. Correlation between ectopic beats and ST depressions during and after exercise in 123 men of different ages

			Age group (yrs)					
			30—39	40—49	50—59	60—69	70—79	80—83
			No. of subjects					
			48	24	14	21	12	4
VEB	ST	I-II	2	2	—	5	1	2
		III	—	2	—	1	1	—
		IV V	1	—	3	1	2	—
No VEB	ST	I-II	38	15	10	9	4	—
		III	6	4	1	3	1	1
		IV V	1	1	—	2	3	1
SVEB	ST	I II	—	2	1	5	2	2
		III	—	—	—	—	2	—
		IV V	—	—	2	—	3	1
No SVEB	ST	I II	40	13	9	9	3	—
		III	6	6	1	4	—	1
		IV V	2	3	1	3	2	—
No EB	ST	I II	38	14	8	6	3	—
		III	6	4	1	3	—	1
		IV V	1	1	—	2	1	—

EB = Ectopic beats. VEB = Ventricular EB. SVEB = Supraventricular EB. ST = Classification of ST-segment, see table III.

Cases with QRS changes at rest. The case with right bundle-branch block had positive T waves at rest but negative T waves in leads CR_4—CR during and after exercise.

The case with left bundle-branch block exhibited at rest a horizontal ST segment without depression in CR_4 and during and after exercise a sagging ST depression. Of the ten cases with extreme left axis deviation (— 30 to — 90°) eight had normal ST segment in the exercise test, the other two being assessed as suspected abnormal or abnormal (age groups 70—83). One of the latter had also abnormal supraventricular ectopic beat and ventricular ectopic beats. Three of the cases with normal ST segment had abnormal or suspected abnormal supraventricular ectopic beats (age groups 60—83), one of them abnormal ventricular ectopic beats as well.

Repeated electrocardiographic recordings. In 31 subjects above 60 years of age the ECG was studied repeatedly. The ECG at rest generally showed no change from the first recording. The total classification of the second ECG at rest was thus changed in only four cases (no ventricular ectopic beats recorded in two cases, lower degree of ST change in one case, and slight prolongation of the P—R interval in one case).

No systematic differences in the electrocardiographic findings were observed during the second exercise test in the sitting position. The differences in classification are given in table VII and

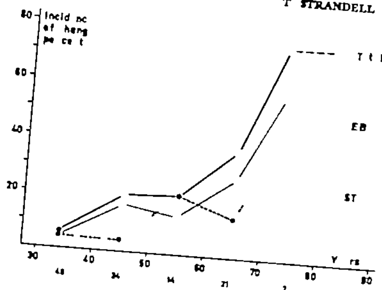


Fig 1 Incidence of individuals with ECG findings classified as abnormal or suspected abnormal during exercise test in different age groups
n = number of subjects,
ST = individuals with ST depressions,
EB = individuals with ectopic beats,
Total = individuals with ST depressions and/or ectopic beats.

Table V Correlation between T waves and ST depressions during an exercise test in 123 men aged 30—83 years (classification see table III)

Classific.	T					
	I	II	III	IV	V	Total no.
ST						
I	55	5	—	—	—	60
II	25	5	—	—	—	30
III	11	2	5	—	—	18
IV	6	2	2	—	—	10
V	2	1	1	1	—	5
Total no.	99	15	8	1	—	123

cases without ventricular ectopic beats (exact testing of two-by two contingency tables (41)) But as the adjacent age groups showed no similar correlation between ventricular ectopic beats and ST depression the observation appears to be uncertain. There was no correlation between supraventricular ectopic beats and the degree of ST depression. No difference has been observed between different types of ectopic beats, either ventricular ectopic beats or supraventricular ectopic beats. No connection between ST changes and ectopic beats, either ventricular or supraventricular was observed in the subjects above 60 years of age who were studied by repeated exercise tests (see below)

Comparison between changes during and after exercise Most ectopic beats were recorded during exercise, only a few after exercise. Twenty-three subjects showed ectopic beats during exercise, classified as abnormal or suspected abnormal, but in only four of them were similar changes recorded also after work. This is in contrast to the abnormal or suspected abnormal changes in ST which were recorded solely during exercise in four subjects (two in the 50—59 and two in the 70—79 age group) during and after exercise in one (70—79 age group) and after exercise in eleven. If tracings had been obtained only after and not during exercise, the incidence of subjects with abnormal or suspected abnormal changes during the exercise test would have been 11 instead of 31 in this material e. g two-thirds of the cases with these findings would have been unrecognized (in most cases ectopic beats)

Table VI. Correlation between ectopic beats and ST depressions during and after exercise in 123 men of different ages

			Age group (yrs)					
			30—39	40—49	50—59	60—69	70—79	80—83
			No. of subjects					
			48	24	14	21	12	4
VEB	ST	I–II	2	2	—	5	1	2
		III	—	2	—	1	1	—
		IV V	1	—	3	1	2	—
No VEB	ST	I–II	38	15	10	9	4	—
		III	6	4	1	3	1	1
		IV V	1	1	—	2	3	1
SVEB	ST	I II	—	2	1	5	2	2
		III	—	—	—	—	2	—
		IV V	—	—	2	—	3	1
No SVEB	ST	I II	40	15	9	9	3	—
		III	6	6	1	4	—	1
		IV V	2	1	1	3	2	—
No ES	ST	I II	38	14	9	6	3	—
		III	6	4	1	3	—	1
		IV V	1	1	—	2	1	—

ES = Ectopic beats. VEB = Ventricular ES. SVEB = Supraventricular ES. ST = Classification of ST-segment, see table III.

Cases with QRS changes at rest. The case with right bundle-branch block had positive T waves at rest but negative T waves in leads CR_4—CR during and after exercise.

The case with left bundle-branch block exhibited at rest horizontal ST segment without depression in CR and during and after exercise a sagging ST depression. Of the ten cases with extreme left axis deviation (— 30° to — 90°) eight had normal ST segment in the exercise test, the other two being assessed as suspected abnormal or abnormal (age groups 70—83). One of the latter had also abnormal supraventricular ectopic beats and ventricular ectopic beats. Three of the cases with normal ST segment had abnormal or suspected abnormal supraventricular ectopic beats (age groups 60—83) one of them abnormal ventricular ectopic beats as well.

Repeated electrocardiographic recordings. In 31 subjects above 60 years of age the ECG was studied repeatedly. The ECG at rest generally showed no change from the first recording. The total classification of the second ECG at rest was thus changed in only four cases (no ventricular ectopic beats recorded in two cases, lower degree of ST change in one case, and slight prolongation of the P—R interval in one case).

No systematic differences in the electrocardiographic findings were observed during the second exercise test in the sitting position. The differences in classification are given in table VII and

Table VII Differences in degree of classification of ST segment (ST) ventricular ectopic beats (VEB) and supraventricular ectopic beats (SVEB) when performing repeated exercise tests in sitting and supine positions (classification see table III)

	Second—first exercise test in sitting position				Exercise test supine — — first exercise test sitting			
	Age groups (yrs)				Age groups (yrs)			
	60–69	70–79	80–83	60–83	60–69	70–79	80–83	60–83
	No. of subjects				No. of subjects			
	15	8	4	27	15	8	4	27
ST								
+2	—	—	—	—	2	2	—	4
+1	1	2	—	3	4	2	—	6
0	12	5	3	20	8	3	2	13
−1	2	1	1	4	1	—	1	2
−2	—	—	—	—	—	—	1	1
						*		
VEB								
+4	—	—	1	1	—	1	—	1
+3	—	—	—	—	—	—	1	1
+2	—	—	1	1	—	1	1	2
+1	2	—	—	2	2	1	—	3
0	11	6	1	18	12	5	1	18
−1	—	1	—	1	—	—	—	—
−2	1	1	—	2	—	—	—	—
−3	1	—	—	1	—	—	—	—
−4	—	—	1	1	1	—	1	2
SVEB								
+4	2	—	—	2	3	1	1	5
+3	—	—	—	—	—	3	—	3
+2	—	—	—	—	1	1	—	2
+1	1	1	2	4	3	2	—	5
0	9	5	—	14	7	—	2	9
−1	3	1	1	5	1	—	—	1
−2	—	—	1	1	—	—	1	1
−3	—	—	—	—	—	—	—	—
−4	—	1	—	1	—	1	—	1

* $P < 0.05$, $P < 0.01$ where P indicates the probability that the differences are caused by random factors.

indicate the error of the method. The reproducibility of the ST changes was rather good, but of the ectopic beats poor especially for the supraventricular ectopic beats. This is at least partly due to the short periods of electrocardiographic recording used, combined with the intermittent appearance of the ectopic beats during an exercise test.

The most marked changes for each subject during the repeated study at rest and during exercise in the sitting and supine positions are seen in table VIII. All but 4 subjects in the ages 60—69 years had shown ectopic beats on at least one occasion, and above 70 years of age only 2 out of 14 had no ectopic beats classified as abnormal. Two cases of short showers of paroxysmal supraventricular tachycardia were recorded one with 58 beats at a frequency of 208/min. during exercise, the other 29 beats at a frequency of 180/min. after exercise.

Table VIII. Incidence of ECG changes in different age groups on repeated examination at rest and during exercise tests. Each subject is represented by the most marked changes in the different groups (classification, see table III)

	Classific.	Age groups (yrs)		
		60—69	70—79	80—83
		No. of subjects		
		16	11	4
Total assessment	I	—	—	—
	II	1	—	—
	III	4	1	—
	IV	—	—	1
	V	11	10	3
ST T	I	2	1	2
	II	3	1	—
	III	6	3	1
	IV	4	1	1
	V	1	5	—
Ectopic beats				
Total	I	4	—	—
	II	3	1	1
	III	1	—	—
	IV	—	—	—
	V	8	10	3
Ventricular	I	10	5	1
	II	—	—	—
	III	1	1	—
	IV	—	—	—
	V	5	5	3
Supraventricular	I	3	—	—
	II	4	2	1
	III	1	—	1
	IV	—	—	—
	V	5	8	2

Effect of body position during exercise. The electrocardiographic changes recorded during the exercise test in the supine position were significantly more marked than in the first exercise test in the sitting position (table VII) although the final heart rate during exercise was significantly lower on an average 13 beats/min. The final load was on an average 134 kpm/min. lower in the supine position. The changes in classification are significant regarding the supraventricular ectopic beats and regarding the ST changes in the 60—79 age groups. In 8 cases the highest class of supraventricular ectopic beats was recorded already at the first load in the supine position (in one case auricular fibrillation which was converted to sinus rhythm 4 min. after work and in four cases 2 or more ectopic beats in a series). In 10 cases the highest class was recorded at a later stage of the exercise test.

Discussion

MATERIAL

All transversal studies of different age groups are attended by problems of selection which render conclusions con-

Table VII. Differences in degree of classification of ST segment (ST), ventricular ectopic beats (VEB) and supraventricular ectopic beats (SVEB) when performing repeated exercise tests in sitting and supine positions (classification see table III)

	Second—first exercise test in sitting position				Exercise test supine— — first exercise test sitting			
	Age groups (yrs)				Age groups (yrs)			
	60–69	70–79	80–83	60–83	60–69	70–79	80–83	60–83
	No. of subjects				No. of subjects			
	15	8	4	27	15	8	4	27
ST								
+2	—	—	—	—	2	2	—	4
+1	1	2	—	3	4	2	—	6
0	12	5	3	20	8	3	2	13
−1	2	1	1	4	1	—	1	2
−2	—	—	—	—	—	—	1	1
						*		
					**			
VEB								
+4	—	—	1	1	—	1	—	1
+3	—	—	—	—	—	—	1	1
+2	—	—	1	1	—	1	1	2
+1	2	—	—	2	2	1	—	3
0	11	6	1	18	12	5	1	18
−1	—	1	—	1	—	—	—	—
−2	1	1	—	2	—	—	—	—
−3	1	—	—	1	—	—	—	—
−4	—	—	1	1	1	—	1	2
SVEB								
+4	2	—	—	2	3	1	1	5
+3	—	—	—	—	—	3	—	3
+2	—	—	—	—	1	1	—	2
+1	1	1	2	4	3	2	—	5
0	9	5	—	14	7	—	2	9
−1	3	1	1	5	1	—	—	1
−2	—	—	1	1	—	—	1	1
−3	—	—	—	—	—	—	—	—
−4	—	1	—	1	—	1	—	1

* $P < 0.05$ $P < 0.01$ where P indicates the probability that the differences are caused by random factors.

indicate the error of the method. The reproducibility of the ST changes was rather good but of the ectopic beats poor especially for the supraventricular ectopic beats. This is at least partly due to the short periods of electrocardiographic recording used, combined with the intermittent appearance of the ectopic beats during an exercise test.

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Table VIII. Incidence of ECG changes in different age groups on repeated examination at rest and during exercise tests. Each subject is represented by the most marked changes in the different groups (classification, see table III)

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		No. of subjects		
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	III	4	1	—
	IV	—	—	1
	V	11	10	3
ST T	I	2	1	2
	II	3	1	—
	III	6	3	1
	IV	4	1	1
	V	1	5	—
Ectopic beats				
Total	I	4	—	—
	II	3	1	1
	III	1	—	—
	IV	—	—	—
	V	8	10	3
Ventricular	I	10	5	1
	II	—	—	—
	III	1	1	—
	IV	—	—	—
	V	5	5	3
Supra-ventricular	I	5	—	—
	II	4	2	1
	III	1	—	1
	IV	—	—	—
	V	5	9	2

Discussion

MATERIAL

All transversal studies of different age groups are attended by problems of selection which render conclusions con-

cerning the longitudinal changes with age difficult or impossible. The present material is highly selected in that the older age groups increasingly represent a positive selection of individuals who are still alive and despite their age are clinically healthy

All individuals below and some above 60 years of age were selected at random from the Stockholm population. As two selections have taken place, one from 1954—55 with 68 % participation (cases with serious electrocardiographic changes at rest were then regarded as not healthy) and one from 1958—59 with 69 % partic ipation this part of the material represents only a random sample from about 48 % of the original random selection from the Stockholm population Ac cordingly not even this part of the ma terial can be considered representative of Stockholmers in general.

Most of the material above 60 years of age was not selected randomly and probably represents a part of the healthy population that is more interested in physical activity than the average subject. In the age group 70—83 the effect of this probable selection cannot be studied but in the 60—69 age group a comparison can be made between the twelve random ly selected individuals and the ten others No differences of any significance ($p > 0.05$) were here found regarding age weight height, degree of physical activity heart rate response during submaximal work, or electrocardiographic changes observed

THE ORTHOSTATIC TEST

In old men the reduction of the cardiac output on changing from the supine to the sitting position at rest is less pronounced than in young (17) as also the increase in the pulse rate. Accordingly the very much rarer orthostatic electrocardiographic changes in the higher age groups appear to correspond to less pronounced circulatory changes on change of position (cf ref. 41)

THE EXERCISE TEST

For evaluation of the significance of electrocardiographic changes during or after work, the degree of loading is of essential importance. Changes occurring during strenuous or maximal exercise may be absent or less pronounced during lighter exercise. The electrocardiographic changes observed during and after essentially maximal exercise, as in the present investigation, can therefore not be directly compared with those observed after other forms of exercise test. In the Master two-step test the effective work done by an ordinary individual of 40 years of age during 1 1/2 minutes (single test) or 3 minutes (double test) corresponds to about 480 kpm/min. (12) The average final level of work in the present study fell with rising age from the equivalent of about 1 000 kpm/min. in 6 minutes at 30—39 years of age to slightly above 600 kpm/min at 80—83 years. The 50—69 age groups probably worked nearer their maximum than the younger men, but it is hardly likely that in any age group the mean final heart rate was more than 10 beats per minute below the maximal heart rate during bicycle exer cise.

Significance of ST depressions The dif ference between the "ischaemic" horizontal or downward sloping (sagging) ST-segment depressions and the junc tional upward sloping ST depressions as sign of heart disease has been repeatedly stressed (4 7 15 42, 50 61 a. o.)

This difference has been found to be statistically established in follow up

studies by means of electrocardiographic recordings (leads I II III V_{1-6}) after the Master double two-step test (44) The cases with "ischaemic" ST-segment depressions after work had a 7 times higher mortality in coronary artery diseases than those with normal ECG A later analysis of the material (45) showed the mortality rate in coronary diseases to be directly correlated to the degree of "ischaemic" ST-segment depression measured in millimetres, whereas the mortality was not elevated either among cases with junctional ST depressions nor among those with arrhythmias after exercise. It was therefore considered that the latter findings could be assessed as normal. But it has also been reported (6) that the morbidity in coronary disease is elevated also in clinically healthy men aged 23—74 years with junctional ST depression after the double Master two-step test of 1.5 mm or more (leads I II V_{4-6}) in contrast to cases with less pronounced junctional ST depression. Depression of the ST junction is observable during or after mild exercise in patients showing "ischaemic" depression of the ST segment after strenuous exercise (3 49, 59) and both forms of depression can be prevented by nitroglycerine medication before the exercise 49) The relative Q—T duration and the length of the junctional ST depression are also considered to be of importance in assessing its significance (38)

Apart from cases of cellular hypoxia and coronary insufficiency ST depressions may be seen in other conditions such as electrolyte imbalance, hormonal disturbances haemoglobin deficit or blockage or enzyme system blockage (28) In the present study of clinically healthy men there was no case of pronounced anaemia. Nervous symptoms as in neurocirculatory asthenia (32) which might have predisposed to electrocardiographic changes via increased sympathetic tone or hyperventilation and alkalosis were not observed and in the higher age groups in which ST depressions were most common orthostatic electrocardiographic changes were rare. Whether a cerebral anoxia, especially in conjunction with the exercise test, may have had an influence on the various electrocardiographic changes in the high age groups is impossible to say (26 33)

There is thus no reason to suppose that the high incidence of ST depressions observed in the high age groups is explainable by extracardiac influences. These ST depressions are then probably indicative of a cellular hypoxia, and it seems natural to relate this to the increased incidence and severity of narrowing of the coronary vessels with age in clinically healthy men (11 34 47 62) Coronary sclerosis is also thought to give rise to hypertrophy of the heart (62) A parallelism within different areas of population has been earlier demonstrated (53) between other electrocardiographic changes with age (decline of $\sum T$) and the narrowing of the coronary artery lumen with rising age.

In the present study the cases with abnormal or suspected abnormal ST depressions in the exercise test did not exhibit any marked depression of the T wave, and in only one case was there an iso-electric T wave after exercise. In a group of men with angina pectoris examined in a similar manner (50) on the other hand, a diphasic or negative T wave was present in at least half of the cases. The difference is probably attributable to the fact that the angina pectoris cases also displayed more marked

ST depressions and need not indicate different mechanisms of origin in the various materials.

The incidence of ST depressions during or after exercise classified as abnormal or suspected abnormal increased in the present material from 4 % in the 30—39 to 39 % in the 70—79 age group In the younger and intermediate age groups the findings accord closely with the results from earlier studies with similar exercise tests (2 3 15 16) in which however the subjects did not consist solely of clinically healthy individuals After 3 minutes of fairly strenuous work on a bicycle ergometer (6) on the other hand a higher incidence of "ischaemic ST depressions was found in the ages 17—64 years, most of which were recorded during the exercise — higher incidence of T wave inversions and lower incidence of ectopic beats. Apart from the varying composition of the materials the differences may be due to the varying types of exercise test.

Significance of T-wave changes That there is a correlation between changes of the T wave in the resting ECG and coronary disease has been illustrated in studies of large numbers of subjects Thus an increased mortality and incidence of infarctions have been reported in clinically healthy men aged 40—69 years with T wave inversion and the same was true of the cases with minor (flat isoelectric) T wave changes (27) Other reports also stress the importance of T wave changes at rest (39)

As regards the significance of T wave changes in exercise tests, most authors (e. g 5 6 8 30 35 48 49 61) state that an isolated diphasic or inverted T after exercise is to be regarded as abnormal or probably abnormal. Follow up studies have, however shown no significant effect of this electrocardiographic finding on the morbidity or mortality in coronary disease (8, 45).

In the present investigation the cases with flattening of the T wave in resting ECG did not exhibit more marked ST depressions in the exercise test than the cases with normal T wave at rest.

Ectopic beats During and after exercise the incidence of supraventricular ectopic beats increased in the present investigation with rising age, especially after 40—50 years. The same has been reported for supraventricular ectopic beats at rest (53) Supraventricular ectopic beats in a series were only seen in the highest age groups. These cases did not exhibit more marked ST depressions than the remainder It may be of interest to note that post mortems of hospital patients with auricular fibrillation who have died after 70 years of age have not revealed a higher incidence of major coronary disease than among the cases of sinus rhythm (46)

It has been pointed out that ventricular ectopic beats with long VAT in the extremity leads at rest is more often combined with an abnormal tracing in other respects than cases with ventricular ectopic beats with short VAT (21) The origin of the ectopic beat is also considered to be a significant factor and it has been reported that in myocardial infarction and hypertonia the ectopic beat usually derives from the left ventricle (60) The incidence of ventricular ectopic beats at rest increases with age (53) especially of ectopic beats deriving from the right ventricle (QRS pattern of left bundle-branch block) (22) In the present material no significant correlation between ventricular ectopic beats during and after exercise and ST depressions was found except in the ages 50—59 years. This might still have been by

chance as in another study of men aged 50—70 years no correlation was found (4). No assured difference has been observed between different types of ventricular ectopic beats.

Opinions are divided as to the significance of ventricular ectopic beats during or after exercise (cf. 30). Isolated or moderately frequent ectopic beats after exercise have not proved significantly to affect the incidence of coronary disease (8, 45). For multifocal ectopic beats or ectopic beats in series or bigeminy the observations in follow-up studies are probably too few for reliable assessment.

Cases with QRS changes at rest. In the 10 individuals with extreme left axis deviation (≤ —30°) of whom 9 fulfilled the criteria for periinfarction block (18) there was not an increased incidence of ST—T depressions or ectopic beats at rest or in the exercise test. Postmortem studies of cases with extreme left axis deviation (10, 18) on the other hand, have revealed high incidences of infarctions, especially anterolateral, and of myocardial fibroses. The significance of this electrocardiographic change must be left to coming follow-up studies.

Effect of body position during exercise. The higher incidence of supraventricular ectopic beats in exercise tests in the supine position is probably due to the high filling pressure recorded under these circumstances in old men (17). The increased degree of ST depression in the supine position may presumably be attributed to the difference in the central circulation. For even in old persons the stroke and minute volumes during exercise are higher in the supine than in the sitting position (17) and the filling pressures for the right and left ventricles — which are high during exercise in the supine position — are lower in the sitting position. At the same time the aortic pressure is unchanged or rather lower in the supine position, for which reason the perfusion pressure over the coronary vessels should be lower during supine exercise. Since the heart rate during exercise is essentially unaffected by the body position, this should imply impaired conditions for perfusion in the supine position at essentially the same stroke work for the left ventricle (stroke volume × pressure increase).

CLASSIFICATION OF ELECTROCARDIOGRAPHIC CHANGES

Until we possess a detailed knowledge of the significance of different electrocardiographic findings at different ages and in the two sexes any system of classification must be open to criticism and the different classes of abnormality cannot have the same significance. The scale employed in the present study for the evaluation of ST depressions finds support in the follow-up studies published hitherto. But no investigation has yet thrown light on the question whether a certain degree of ST depression has the same prognostic significance in different age groups. As regards ectopic beats during exercise there are at present no follow-up studies of clinically healthy individuals with this finding, nor for healthy individuals with peri-infarction block. Whether the classification employed for these findings is appropriate and provides prognostic or otherwise important information can, therefore, not be stated with certainty at this time.

It may perhaps seem that an electrocardiographic interpretation which yields so high an incidence of abnormal findings in exercise tests of clinically healthy men at high ages as in the

present study must lack practical significance for an individual assessment at these ages. It has been pointed out (53) that the electrocardiographic evaluation should instead be based on the findings in apparently healthy people in different age groups. The aim would thus be to give the electrocardiographic diagnosis the same diagnostic significance as a clinical examination!

But this would be to deprive oneself of the possibility of gaining information concerning changes which have not yet yielded clinical symptoms Whether electrocardiographic findings at rest and during and after exercise in healthy old persons can provide this prognostic information as in younger people, has not yet been definitely proved Electrocardiographic changes at rest in individuals aged 60—90 years, however seem to have some significance for their survival (14) Furthermore, at ages of 50—64 years the recording of changes in the exercise ECG seems to anticipate the development of clinical symptoms (4) For the individual electrocardiographic diagnosis of old persons it would seem advisable to evaluate the changes both in relation to the findings in young, clinically healthy persons and to the findings in clinically healthy persons in the age groups concerned

Summary

One hundred and twenty-six clinically healthy men aged 30—83 years mostly randomly selected, have been studied by electrocardiographic recording at rest in the supine and standing positions and during and after exercise. The tracings in all age groups have been judged by the same standards.

The exercise was performed on a bicycle ergometer in the sitting position with successively increasing loads up to maximal or near maximal intensities. Only one subject (73 years) reported angina pectoris. The mean final load decreased from 1 004 kpm/min. in the 30—39 to 634 kpm/min. in the 80—83 age group and the mean final heart rate during exercise correspondingly from 176 to 134 beats per min.

Left axis deviation —30 — —90 of the type called peri infarction block was found in 9 subjects, being most frequent in the highest age groups. These subjects had the same incidence of electrocardiographic changes during work as the remainder of the material.

Orthostatic ST—T changes were rather uncommon and were recorded in 14 per cent in the 30—39 and 2 5 per cent in the 40—83 age group

The reproducibility of the electrocardiographic changes during an exercise test was good regarding ST depressions but poor regarding ectopic beats. This may at least partly be due to the intermittent recordings of the ECG during and after exercise.

The incidence of subjects with electrocardiographic changes classified as abnormal or suspected abnormal during an exercise test increased from 6 per cent in the 30—39 to 75 per cent in the 70—83 age group ST depressions with this classification increased from 4 to 38 per cent supraventricular ectopic beats with this classification from 0 to 31 per cent, and similarly classified ventricular ectopic beats from 4 to 31 per cent.

Most ectopic beats were recorded during exercise and not after whereas the ST depressions generally were most marked after exercise.

No significant correlation was found between ectopic beats and ST depressions during and after exercise.

The T-wave changes during the exercise test were not as frequent as the ST depressions and were generally seen in combination with the same or a higher degree of ST depressions. No negative T wave was found during or after exercise except in a case with bundle-branch block.

During and after exercise in the supine position, significantly more marked ST depressions and supraventricular ectopic beats were recorded than in the sitting position. This may be explained by the differences in central circulation between the two postures.

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From the University Medical Clinic B, Rikshospitalet, Oslo, Norway

The Renal Metabolism of Citric Acid

By

Erling Knude Brodwall and Helge Laake

Normally cyclic exchange of citrate (Ci) takes place in the organism. Plasma is the transport medium, and there is a considerable uptake of citrate in the kidneys and liver. Isotope studies with citrate — C^{14} (2) have confirmed this and proved that the tagged citrate is incorporated mostly into protein and lipid. Three hours after injection, 90 % of the radioactivity was found in the respiratory CO_2 and in urinary citrate.

Of the endogenous citric acid (Ci) passing through the kidneys, a small fraction is excreted in the urine. Animal experiments on the arterio-venous (a—v) concentration gradient in the kidneys (4, 8) have shown that the capacity to metabolize Ci is very great. Mårtensson (8) maintained that the renal oxidation of Ci is the limiting factor in the tubular reabsorption of Ci.

There has been comparatively little research done on the renal excretion of Ci, and the capacity of the human kidney to metabolize the endogenous Ci has not been thoroughly investigated.

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Present investigation and results

In our clinic we have studied the renal metabolism of Ci by catheterization of the renal vein in a clinical material. The analyses for citric acid were carried out using the pentabromacetone method, the renal plasma circulation was determined by PAH clearance for 2 or 3 ten-minute periods, and the glomerular filtration by inulin clearance. The amount of Ci metabolized in the kidneys is the difference between the Ci conveyed to the kidneys and the sum of the Ci found in the renal vein and in the urine.

In our normal group of 9 cases (table I) the average a—v difference was 0.5 mg/100 ml, and in a larger group of normal cases (6) the Ci level in the blood was 2.05 (± 0.26) mg/100 ml. Table I shows that in normal individuals an average of 2.7 mg Ci/min. is metabolized, the individual variations being relatively large, from 2.11 to 4.1 mg/min. If the amount of Ci metabolized is compared with the amount filtered and reabsorbed (the difference between the Ci filtered and that excreted in the urine) it is found that

Table I Normal

Citric acid

mg/100 ml plasma	A V diff (mg/100 ml)	Metabolized (mg/min)	Filtered (mg/min)	Excreted in urine (mg/min)	Inulin clearance (ml/min)	RPF (ml/min)	RBF (ml/min)	E_{PAH} (%)
1.5	0.46	2.2	1.5	0.62	100	605	931	86
1.9	0.63	3.1	3.2	0.54	166	574	998	88
1.2	0.41	2.2	1.33	0.34	111	619	1 032	87
2.02	0.42	2.5	2.0	0.28	99	663	1 101	88
1.85	0.45	2 7	1.5	0.65	120	748	1,362	86
2.6	0.57	3.2	3.4	1.6	131	840	1,555	90
1.95	0.36	4 1	2.7	0.95	139	1 117	1,959	81
1.9	0.33	2.11	2.1	0 74	111	636	1 051	91
2.3	0.55	2.37	2 03	0 46	88	515	896	87

Table II Acetazolamide block

Citric acid

mg/100 ml plasma	A-V diff. (mg/100 ml)	Metabolized (mg/min)	Filtered (mg/min)	Excreted in urine (mg/min)	Reabsorb. (%)	Inulin clearance (ml/min)	RPF (ml/min)	RBF (ml/min)	E_{PAH} (%)
2.39	0.25	1 15	2.1	0.52	80	88	669	1 061	80
1.34	0.2	1 0	1 1	0.34	73	80	678	1,256	79
1.8	0.33	1.2	1.36	0 4	70	77	468	739	83
1.6	0 11	0.4	1.53	0.4	74	98	747	1,264	82
1.0	0.2	0.94	0.9	0.38	61	88	615	1,206	84
1 1	0.24	1.32	0.87	0.24	72	81	551	889	89

the human kidney metabolizes more citric acid per unit of time than that re absorbed from the tubular urine with a normal Ci level in the blood

Investigations on Ci metabolism with isotopes in rats have shown that fluor acetate inhibits acetate oxidation in the liver and kidneys (2) Several of the steps in the citric acid cycle are dehydrogenation processes requiring oxygen. We have studied the renal metabolism of Ci (table II) using acetazolamide blockade, the most suitable method for clinical tests. The amount of Ci metabolized is reduced by this blockade, and similarly the a—v Ci difference, which in these observations had an average value of 0.22 mg/100 ml. In the normal cases the amount of Ci metabolized per minute was 2 7 mg and with acetazolamide blockade it was 0 93 mg Depending on the degree of blockade, the amount of Ci metabolized was greater than similar to or less than the amount reabsorbed. The Ci reabsorption percentage in our block ade tests varied from 61 to 80 % (average

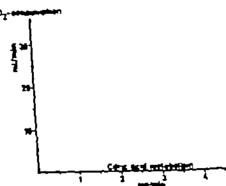


Fig. 1. The relation between the renal citric acid metabolism and oxygen consumption in normal kidney

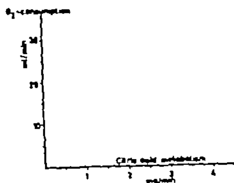


Fig. 2. The relation between the renal citric acid metabolism and oxygen consumption before (O) and during (+) acetazolamide inhibition.

Table III. Nephropathies

Citric acid

mg/100 ml plasma	A-V diff (mg/100 ml)	Metabolized (mg/min)	Filtered (mg/min)	Excreted in urine (mg/min)	Inulin clearance (ml/min)	RPF (ml/min)	RBF (ml/min)	E_{PAH} (%)
1.9	0.35	1.7	2.4	0.57	130	848	1,502	78
1.7	0.40	1.15	1.12	0.43	67	411	658	83
2.5	0.37	1.73	1.43	0.76	61	426	645	83
2.65	0.43	1.1	2.84	0.90	107	328	478	79
1.2	0.23	0.51	0.54	0.50	47	353	568	82
2.4	0.20	0.30	1.54	0.60	64	459	693	64

72 %) which approximately corresponds to the normal Ci reabsorption.

Taggart (9) maintained that the utilization of such substrates, which feed directly into the tricarboxylic acid cycle re-emphasizes the predominantly aerobic nature of renal metabolism. Citrate synthesis and metabolism take place in the tricarboxylic acid cycle. The renal oxygen consumption has been investigated and correlated with the Ci metabolism in normal individuals (fig. 1) and in cases with acetazolamide blockade (fig. 2). The normal oxygen consumption in the kidney is approximately 20 ml/min. These blockade tests show that the oxygen consumption is reduced in parallel with the reduction of Ci metabolism.

In our department it has previously been shown that the endogenous Ci clearance is reduced in acute and chronic nephritis (6). The renal metabolism of Ci has now been investigated in different types of nephropathy (table III). In disturbed renal function there was marked reduction of the renal metabolism of Ci

Table I Normal

Citric acid

mg/100 ml plasma	A-V diff. (mg/100 ml)	Metabolized (mg/min)	Filtered (mg/min)	Excreted in urine (mg/min)	Inulin clearance (ml/min)	RPF (ml/min)	RBF (ml/min)	E_{PAH} (%)
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Citric acid

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1.8	0.33	1.2	1.36	0 4	70	77	468	739	83
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1.0	0.2	0.94	0.9	0.38	61	88	615	1,206	84
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A large part of the total oxygen consumption in the kidney is used in sodium transport (1, 5) but the renal synthesis and metabolism of Ci is also of an aerobic nature. During acetazolamide blockade the sodium transport from the tubular urine is reduced, and this may be a contributory cause of the reduced oxygen consumption. With the technique used it is not possible to obtain a quantitative idea of the oxygen consumed in the sodium transport or of the enzymatic processes in the metabolism of citric acid.

It must be supposed that the tubular reabsorption of Ci is normally an active energy-demanding transport mechanism. During enzyme blockade with acetazolamide or with organic lesions in the tubules the transport is inhibited, and Ci is probably able to diffuse from the tubular lumen into the cells of the tubules. Milne et al. (7) postulated that there is a dynamic equilibrium between the Ci in the tubular lumen and that in the cells of the tubules, and Grollman et al. (3) maintained that diffusion of Ci probably takes place into the tubular cells from the peritubular space, and that this process is not influenced by the presence of other tricarboxylic acids.

In Grollman et al's (3) acetazolamide blockade experiments in dogs with metabolic alkalosis the Ci clearance was 0, and this was explained by complete reabsorption of the filtered Ci. Our investigations showed that the reabsorption of Ci was unchanged during acetazolamide blockade. Both in the blockade experiments and in nephropathies it must be assumed that there is some degree of diffusion of Ci from the tubular urine, and this may be the cause of the reduced clearance values.

There are no clinical clearance studies demonstrating tubular secretion of Ci. In dogs it has been shown that Ci is not secreted in the tubules (3) but we are not justified in assuming that experimental physiology and clinical research will lead to analogous conclusions.

On account of the low Ci content of erythrocytes we have calculated the renal uptake of Ci on the basis of the renal plasma flow. In assessing the method used for calculating the renal metabolism of Ci, one should bear in mind that the reliability of this estimate is limited and pre-supposes a steady state as regards the renal plasma flow, the a—v concentration gradient and the tissue metabolism (10). Several previous investigations have shown relatively large fluctuations in the plasma flow. In the investigations reported here the plasma flow was relatively constant during the periods of the experiments.

Summary

By catheterization of the renal vein in normal individuals an arterio-venous (a—v) concentration gradient of citric acid of 0.5 mg/100 ml was found. The human kidney metabolizes an average of 2.7 mg citric acid per minute. The metabolized citric acid includes the reabsorbed citric acid and that synthesized in the kidney.

During acetazolamide blockade the a—v concentration gradient of citric acid and the amount of citric acid metabolized are reduced. The renal oxygen consumption is also reduced.

In different types of nephropathy marked reduction of the renal metabolism of citric acid, the oxygen consumption and of the a—v concentration gradient were found. The reduced turnover of Ci is attributed to reduced activity of tubular enzymes that are involved in the synthesis and metabolism of citric acid.



Fig 3. The relation between the renal citric acid metabolism and oxygen consumption in nephropathia (+) and in normal kidney (O)

and of the a—v concentration gradient. The relation between the Ci metabolism and the oxygen consumption in nephropathies is shown in fig 3

Comments

In animal experiments the Ci renal a—v concentration difference is reported as 0 3 mg/100 ml and Mårtensson (8) found that in rabbits the a—v difference was about 30 % Our experiments showed that in the human kidney the a—v difference was 25 % of the endogenous citric acid level in plasma. Herndon et al s (4) and Grollman et al s (3) studies on the renal citric acid utilization in dogs showed that the amount of citrate removed by the kidney is somewhat greater than that calculated to be filtered, and they concluded that the citric acid metabolized by the kidneys is not derived solely from that taken up from the tubular lumen Our clinical studies showed that the human kidney also metabolizes more Ci per unit of time than that reabsorbed from the tubular urine with a normal Ci level in plasma. Besides tubular reabsorption of filtered Ci synthesis of Ci also takes place in the kidneys, and the total amount metabolized is made up of reabsorbed and synthesized Ci. Tissue analyses on kidney (8) showed that the Ci concentration was identical with or higher than that in the blood.

In acetazolamide blockade experiments in alkalotic dogs it was not possible to demonstrate Ci in the urine with normal plasma Ci concentration. Grollman et al. (3) accepted this as an indication of complete tubular reabsorption of the filtered Ci. Our clinical investigations on the tubular reabsorption of Ci showed that this was normal during acetazolamide blockade. During such a blockade the normal synthesis of Ci is inhibited and the amount of metabolizable Ci is reduced. The reduced turnover of Ci during acetazolamide blockade (table II) may depend on reduction of both synthesis and metabolism of the reabsorbed Ci.

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Gas-mixing in the Lungs

A Study of the Clinical Applicability of the So-called Becklake-index

By

E. Strange Petersen

Uneven ventilation is now a well established factor in human pulmonary pathophysiology (7 15 19) There is therefore among physicians an increasing demand for an objective measure of the degree of uneven ventilation.

The numerous experiments which have been carried out in order to elucidate gas-mixing in the lungs have consisted largely of analyses of the course of nitrogen elimination during oxygen breathing in open circuit systems (9) Several methods have been suggested to measure the degree of uneven ventilation (7 15 19)

Of these, the so-called "lung-clearance index" originally described by Becklake (2) will be discussed in this paper The definition of this index, usually called Becklake-index, is "litres of ventilation required to wash each litre of the functional residual capacity free of nitrogen while breathing oxygen"

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In Becklake's original paper the index was computed as follows

$$\frac{\text{litres ventilation required to wash 90 \% } \mathit{FRC} \text{ free of N}}{90 \text{ \% } \mathit{FRC}}$$

It is, however most frequently used in a slight modification (4)

$$\frac{\text{litres ventilation required to lower } N_2 \text{ conc. in expired air to 2 \%}}{\mathit{FRC}}$$

FRC is the functional residual capacity (this abbreviation will be used throughout this paper)

The clinical applicability of the Becklake-index is apparently excellent. The experimental determination of it is quite simple, being done in the course of determining *FRC* which in several laboratories is a routine measurement. The index is expressed as a single num-

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extent, unevenly ventilated. A model taking into account the simultaneous, parallel occurrence of several wash-out processes would have been more appropriate (12, 23)

That the respiratory frequency cannot be incorporated in the model employed is a further limitation of its use, as the frequency of breathing is likely to be factor of considerable importance in studying pulmonary gas-mixing.

Otis (19) has in model experiments investigated the functional result of stenoses in the air-ways, which with differences in distensability of the pulmonary tissue is a most important cause of uneven ventilation.

Otis placed large rubber balloons of equal size in chamber where the pressure was altered periodically The balloons were connected to each other and to the outside by means of Y-piece in which one of the branches was stenosed.

Application of a negative pressure in the chamber with certain frequency now caused the balloons to be alternately distended and compressed. The ventilation of the 2 balloons was approximately equal at lower frequencies. If however the ventilatory frequency was increased, the 2 balloons came out of phase with each other The higher the frequency the less was the ventilation, relatively and absolutely of the balloon with the stenosed tube.

Gas-mixing in the lungs, as expressed by Becklake-index, t different respiratory frequencies has been little investigated and will be the topic of a later study

Fritts and Lindqvist (13) have determined Becklake-index in healthy subjects with different types of breathing. They found values between 6.0 and 7.8 (mean 7.0) with deep, slow breathing, while with shallow quick breathing they found values from 6.9—13.1 (mean 8.8) This experiment supports the assumption that the frequency of breathing during the experiment is factor of considerable importance when the result of the experiment is used in evaluating the degree of uneven entilation.

It might thus be expected that potential mixing disturbances caused by stenosed air ways tend to disappear during slow and deep breathing, while they are accentuated by frequent and shallow respiration.

Bouhuys et al. (4) have investigated the possible role of the tidal volume for the N wash-out during oxygen breathing. The wash-out pattern of normal subjects was studied. The minute-ventilation was increased 2 and 3-fold by work of varying intensity Only small changes in the frequency of breathing were found during the periods of work. As an increased minute-ventillation causes a relative decrease of the respiratory dead space, more efficient wash-out, as expressed by Becklake-index, was to be expected. The uthors, however found no such changes.

This was confirmed by Grere (16) who showed that increasing the tidal volume from 500 ml to 3,000 ml, at a given dead space, caused no changes in Becklake-index.

In a later work Bouhuys et al. (5) increased the tidal volume in another way by adding an extra dead space in the experimental set-up. It could not be shown, however that the wash-out was more efficient under these experimental conditions, but a marked tendency towards more even ventilation was found. As an explanation of this the authors suggested that expired gas from different parts of the lungs is mixed in the large dead space and inhaled at the following inspiration, thereby contributing to a more uniform concentration of the alveolar gases.

The pulmonary disability in emphysema is most frequently caused by two factors, an increased residual olume and varying degrees of uneven ventilation (9) but may also be caused solely by the increased residual volume (10)

The Becklake-index ppears from theoretical point of view to be satisfactory measure of uneven ventilation in the lungs. The author has therefore experimentally tested its clinical applicability In particular it has been the aim to assess the error of the method and to ascertain whether the Becklake-index may contribute to an understanding of the pathophysiology in emphysema.

Material and methods

In this paper are presented results from experiments with

1) A group of 10 patients with pulmonary emphysema, all in-patients t the department, but not confined to bed t the time of the experiments. The diagnosis was based upon case-history shape and mobility of thorax,

Table I Calculated values of Becklake-index at different levels of functional residual capacity, presuming that volume of tidal air and dead space are kept constant Functional residual capacity tidal air and dead space expressed in ml

Tidal air	Dead space	Becklake index at FRC					
		1,500	2,000	3 000	4,000	5 000	6 000
500	150	5.81	5 79	5.55	5.48	5 44	5.42
300	150	8.14	7.96	7 77	7.68	7.62	7.59
500	250	8.62	8.33	8.03	7.86	7 77	7.69

ber which for clinical purposes is of help in interpreting the N_2 wash-out curve. Moreover it appears because of the introduced correction for *FRC* to be superior to other suggested measures of uneven ventilation (8 14) as a pure index of gas-mixing in the lungs widely independent of the magnitude of *FRC*

Theoretical considerations

Considering the lungs as a bellows which is uniformly ventilated the following equation describing the progressive reduction of alveolar N_2 concentration during oxygen breathing, may de deduced

$$F = F\left(\frac{FRC + V_D}{FRC + V_T}\right)^n$$

In this equation (10 12) F and F represent the alveolar N concentrations, respectively before start of oxygen breathing and after n breaths of oxygen *FRC* is the functional residual capacity V_T the tidal volume and V_D the volume of the respiratory dead space.

Applying the frequently used technique where oxygen breathing is closed at an N conc. of 2 F and F may be regarded as constants, and after rearranging the equation and taking the logarithms we have

$$n = \frac{K}{\ln\left(\frac{FRC + V_T}{FRC + V_D}\right)} = \frac{K}{\ln\left(1 + \frac{V_T - V_D}{FRC + V_D}\right)}$$

As $\ln(1 + x) = x - \frac{x^2}{2} + \frac{x^3}{3} - \frac{x^4}{4}$

and presuming x to be a sufficiently small fraction, we have with fairly good approximation

$$\ln(1 + x) = x$$

Using this, the expression for n may be simplified

$$n = K\left(\frac{FRC + V_D}{V_T - V_D}\right)$$

or

$$n = \frac{K}{V_T - V_D}\ FRC + \frac{K\ V_D}{V_T - V_D}$$

Thus n — with constant V_T and V_D — the equation of a straight line in a coordinate system where n is ordinate and *FRC* is abscissa.

The physiological implication of this is that the number of breaths of oxygen, required to lower the N_2 conc. in the alveolar air down to for instance 2 % is a linear function of *FRC*, supposing that V_T and V_D are constant.

As $(V_T - V_D)$ is the denominator in the coefficient for *FRC*, a reduction of it, whether caused by an increased V_D or a decreased V_T will result in a steeper line as an expression of less efficient ventilation.

FRC and V_D being constant, the equation may be rearranged to

$$n\,(V_T - V_D) = K\,(FRC + V_D)$$

and it is seen that n is inversely proportional to $(V_T - V_D)$

With the symbols employed,

$$\text{Becklake-index} = \frac{n\ V_T}{FRC}$$

and substituting the above mentioned expression for n

$$\text{Becklake index} = \frac{K\,(FRC + V_D)\,V_T}{FRC\,(V_T - V_D)} = \frac{K\ V_T}{V_T - V_D}\left(1 + \frac{V_D}{FRC}\right)$$

This shows that for constant values of V_T and V_D, Becklake-index is a decreasing function of *FRC* when this is expressed in its absolute value Inside physiological limitations, however the variations are small a couple of simple calculations are shown in table I to illustrate this.

The formula employed can only be an expression of a crude model of the true pulmonary conditions. Thus, it is an established fact (21 18) that even normal lungs are to some

A small amount of the expired gas, approximately 20 ml/min., is sucked through a needle-valve into a nitrogen-meter (gas-analyser-model Th. Kyrre) The impulse from this is conveyed by an electronic amplifier to be recorded by a one-channel-writer (Mega watt) The experiment is finished when the N concentration at the end of an expiration is 2 %.

The expired gas, collected in the Douglas bag, is now measured in gas-meter (Bohr experimental-meter 1 liter) and its N conc. is determined.

As oxygen breathing was commenced at the end of normal expiration, FRC and Becklake index may now be computed, taking into account the dead space of the set-up and the N elimination from the tissues during the wash-out (11). The small amount of gas sucked through the nitrogen-meter has not been taken into account in the calculation.

All volumes were corrected to BTPS.

Before each experiment the nitrogen-meter was calibrated with oxygen, room-air and 3 known O_2-N mixtures with N contents of 2, 10 and 14 % respectively The composition of these gas-mixtures and the collected expired air has been checked repeatedly with the Scholander-apparatus (20).

In all subjects 2 identical experiments were made with an interval of at least 20 minutes. In a few cases one of the analyses failed — these are not included in the table of results, as an essential point in this paper was an elucidation of the reproducibility of the determinations.

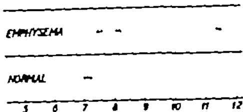


Fig. 1 Becklake-index in normal and emphysematous group. The three triangles in the emphysema-group indicate patients with mild or moderate emphysema.

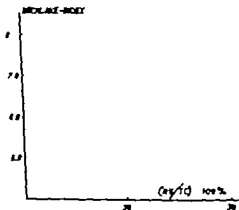


Fig. 2. Relationship between residual volume in % of total capacity and Becklake-index. Normal subjects.

Results and discussion

Tables II and III present the results, in th normal and the emphysematous subjects espectively

Mean values for Becklake-index in the subjects are presented in fig 1

The mean value of the Becklake-index in the normal-group was 6.95 (SD 0.68) In the emphysema-group the mean was 9.23 (SD 1 49) These figures gree with those found by other authors (2, 3 4 6 13)

Concerning the applicability of the Becklake-index in separating normals and subjects with uneven ventilation, the results here presented confirm Becklake s findings (2)

Figs. 2 and 3 are graphical representations of the relation between the residual volume in % of the total capacity and the Becklake-index. The relatively small number of experiments does not justify any definitive evaluation. The results however indicate the presence of a rough linearity This confirms the likely supposition, that subjects with the most severe emphysematous changes, measured

Table II Experimentally found values of the functional residual capacity in ml, the residual volume in % of the total capacity and Becklake-index Normal subjects

Subject	Sex	Age	FRC	RV/TC 100%	Becklake index	(mean)
LS	♀	24	2,682 2,896	25 29	7.5 6.9	7.2
SESP	♂	27	3 410 3 61*	25 27	6.5 6.9	6.7
ILM	♀	22	2 413 2,585	29 30	7.5 8.1	7.8
AVP	♀	25	2,413 2,875	21 27	7.5 7.3	7 4
JBH	♂	26	4 111 4,510	20 24	6.1 5.8	6.0
AL	♂	31	4,552 3,898	29 22	7 4 5.9	6.7
GN	♂	31	3,774 3,458	26 21	5.5 6 1	5.8
EHF	♀	30	2,800 2,792	28 27	6 1 7.9	7 0
KR	♂	26	3,228 3,272	29 29	6.5 7.6	7 1
LA	♀	27	2,293 2 081	28 24	8.3 7.2	7.8

Table III Experimentally found values of the functional residual capacity in ml, the residual volume in % of total capacity and Becklake-index Emphysematous subjects

Subject	Sex	Age	FRC	RV/TC 100%	Becklake-index	(mean)
JBJ	♂	65	3,853 4,366	46 50	7.8 7.3	7.6
LSMP	♂	51	4,925 4,334	59 55	8.7 9.9	9.3
SVC	o	57	7 074 6,986	80 78	10.6 12.2	11.4
RPBP	o	63	4 192 4,080	59 58	13.1 9.8	11.5
JMKH	♂	53	3,566 2,740	50 41	7.6 8.8	8.2
EPL	♂	69	3,550 3,741	56 57	9.3 11.5	10.4
SL	♂	60	3 021 3 192	35 38	7.2 7.8	7.5
SMN	♂	36	3,513 3,562	37 38	7 7 8.5	8.1
AC	♀	58	3,617 3,837	65 67	7 7 9.1	8.4
AEB	♀	63	3,358 3 185	53 51	10.4 9.4	9.9

and X ray appearance of lung borders and lungs. With these criteria, the chief physician of the department placed the patients in 3 clinical grades of severity

grade I = mild emphysema
grade II = moderate emphysema
grade III = severe emphysema.

2) A group of normal subjects, 10 healthy young adults, all belonging to the hospital staff. None of these had ever had heart or lung disease.

Determination of vital capacity volume of expiratory reserve and timed vital capacity was done in all subjects, employing a 9 liter spirometer (Eksspirograf" — Godart Mij hardt) In all subjects belonging to th normal group normal values were found, whereas all the emphysematous subjects exhibited pathological vital capacities and timed vital capacities in varying degrees. Knowing these spirometrically determined volumes and the later determined *FRC*, it was possible to calculate the residual volume and express it in percentage of the total capacity

FRC and Becklake-index were measured in an open circuit system. An experimental set up, essentially identical with that described by Fritz and Lindqvist (13) was used.

In a sitting position, with mouth-piece and nose-peg the subject is connected to a system with 2 double-way-cocks and allowed to breathe room-air at the beginning of the experiment. At the end of a normal expiration the cocks are switched so that the subject now inspires pure oxygen from a flask equipped with a 2 liter balloon and escape-valve The expired gas goes into an empty Douglas bag, previously washed out with oxygen.

termination, and *n* is the total number of experiments.

The standard error s, for all determinations of the Becklake-index in both groups was found to be 0.93 expressed in percentage of the mean 11.5 %.

This standard error is of approximately the same size as the standard deviation in the normal group but smaller than the standard deviation in the emphysema-group. It is the expression of a considerable methodological error

Where mean values of double determinations are presented in tables and figures, the standard error is) 2 smaller

In a search for the cause of the error the expression for the Becklake-index was considered

$$\text{Becklake-index} = \frac{\text{ventilation during the N wash-out}}{FRC}$$

and was computed for the denominator as well as the numerator in the fraction.

The determination of the ventilation during the N wash-out gave $s = 3.61$ 12 1) and the determination of *FRC* gave $s = 251$ (7 0 %)

The standard error was also estimated for each of the two groups, and in all cases found la ger in the emphysema-group. The differences however wer not significant.

Other uthors have found reproducibilities of the Becklake-index determinations of 17 and 20 errors 9—10 % for the *FRC* determinations (3 22) showing the presence of a methodological error of nearly the same size as that found in the present study

It now seems likely that the essential source of the error lies in the numerator

Because of the exponential course of the declining N percentage, it may be difficult to top the experiment t the precise 2 point in spite of the effort to have a uniform experimental procedure. It is therefore likely to happen that either too many or too few respirations are included, and this markedly influences the determination of the total ventilation i.e. the numerator It is far less important for the calculation of *FRC* as this is computed as the product of the ventilation and its N content.

Another possible explanation of the demonstrated error was that oxygen breathing was commenced at the end of a normal expiration. This starting point, however the so-called *resting* respiratory level, is not constant because of changes in the height of the diaphragm, thus potentially causing changes in *FRC* (17) Since the error in the *FRC* determinations is however not very large it may be presumed that the most important source of the error is the one first pointed out.

The importance of maintaining a uniform experimental procedure, concerning the switch-over point to oxygen breathing is stressed by an experiment made by Fritz and Lindqvist (13) They found Becklake-indices of 5.9 and 9.9 when switching over to oxygen breathing respectively at the end of a forced inspiration and at the end of a forced expiration.

In comparing the results in the normal and the emphysematous groups the age of the subjects should be taken into account. The normals in the present study were all 22—32 years old. Of the emphysematous subjects one was 36 the others from 51 to 69 The lungs of older people exhibit, because of decreasing elasticity structural changes similar to those found in emphysematous lungs (1) In agreement with this, with advancing age there is found a not inconsiderable increase of the functional residual capacity which is caused largely by an increase of the

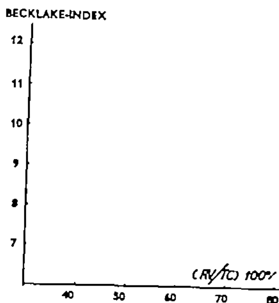


Fig 3 Relationship between residual volume in of total capacity and Becklake index. Emphysematous subjects Triangles indicate patients with mild or moderate emphysema.

Table IV Correlation of clinically estimated degree of emphysema, residual volume in % of total capacity and Becklake-index

Subject	Clin. emphys.	$\frac{RV}{TC}$ 100%	Becklake index (mean)
JBJ	III	48	7.6
LSMP	III	57	9.3
SVC	III	79	11.4
RPBP	III	59	11.5
EPL	III	57	10.4
SMN	III	38	8.1
AEB	III	52	9.9
AC	II	66	8.4
JMKH	I	46	8.2
SL	I	37	7.5

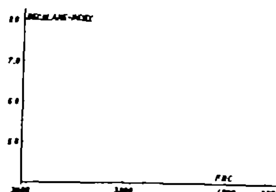


Fig 4 Relationship between functional residual capacity in ml and Becklake-index.

as the residual volume's contribution to the total capacity also exhibit the most pronounced degrees of uneven ventilation

The author has, in agreement with the previously mentioned calculations (table I) found in the normal-group a small decline of Becklake index with increasing *FRC* This is true only when *FRC* is expressed as an absolute value (compare fig 4 with figs 2 and 3) No such relation could be demonstrated in the emphysema-group

In spite of the demonstrated linearity between the size of the residual volume and the Becklake index, the latter did in some cases contribute to a better agreement between the clinical and physiological findings (table IV) It may thus be assumed that subject AC who clinically is among the least disabled of the emphysematous subjects (grade II) and whose residual volume is among the largest measured exhibits but smaller degrees of uneven ventilation. Furthermore it may be assumed that the pronounced clinical disability in subjects RPBP and EPL, whose residual volumes were but moderately increased, is at least partly due to uneven ventilation.

The standard error of the method in the individual experiments has been computed from the formula

$$s = \sqrt{\frac{\sum |m_1 - m|}{n}}$$

where m_1 and m are the results in the 2 experiments comprising the double-de-

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Respiratory Compensation in Metabolic Alkalosis

By

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For the last four decades hyperventilation has been recognized as an important clinical feature of severe metabolic acidosis. The adverse effect of increased alveolar ventilation on a pathologically decreased pH value of plasma has permitted this secondary hyperventilation to be classified as compensatory phenomenon. Furthermore, the fact that e spiratory compensation in metabolic acidosis is never complete suggests that the hydrogen ion itself is triggering the compensatory mechanism. The existence of compensatory breathholding and CO retention in primary metabolic alkalosis thus came as a reasonable inference and as such has found its place in the physiological textbooks and monographs (10 15, 23 31)

So far the few investigations available on this topic have largely been concerned with the mere existence or non-existence of respiratory compensation in metabolic alkalosis. In perfusion experiments Hooker et al. (17) noted that sodium bicarbonate nd hydroxide tended to depress the medullary centers, but further attempts to demonstrate significant hypoventilation in acute experimental alkalosis in animals (chiefly dogs) (13 20 24 25 30) and in man (27) have almost invariably failed, and the positive results advanced by Bramlitt and Hardy (6) seem inconclusive because of inconsistent values obtained by two different methods. However the early clinical literature contains several descriptions of the slow shallow and irregular breathing of infants with pyloric stenosis (1 14 16) and venous acid-base data published by Hartmann (15) include figures for a group of infants with pyloric stenosis the pCO values of whom re clearly elevated. A few further clinical figures for venous blood showing raised pCO levels in metabolic alkalosis have been collected from the literature by Peters and van Slyke (23) More recently single adult cases showing rather pronounced respiratory compensation have been mentioned by Astrup (2) and Møller (22)

It is the purpose of the present paper to demonstrate the existence of compensatory CO retention by a large number of measurements performed on infants with pyloric stenosis and varying degrees of metabolic alkalosis, to define some of the chemical and physiological conditions

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residual volume and only to a lesser extent by an increased expiratory reserve (1 7 19)

A comparison of Becklake indices found in different age-groups has been made by Boye (6) who found no significant changes with advancing age Nor could the same author demonstrate any sex differences in this respect.

Summary

It appears from theoretical considerations that the Becklake index as a measure of uneven ventilation in the lungs is only slightly dependent upon the size of the functional residual capacity When the tidal volume is bigger than 500—1 000 ml a further increase of the tidal volume will cause no or only insignificant changes in index. As the experimental determination is quite simple, the Becklake index apparently has good clinical scope.

The author made N_2 wash-out studies during oxygen breathing in 10 normal and 10 emphysematous subjects Double determinations were made of the functional residual capacity and the Becklake index. The results which are in close agreement with those of other authors have shown that Becklake index offers a good separation between healthy and emphysematous subjects. Moreover in a few of the emphysematous subjects the index has helped elucidate the pathophysiological features causing clinical disability

The author's experiments have revealed a considerable methodological error in the determination of the Becklake index. It seems justified to place the main source of this error in the break-off point of oxygen breathing at a N_2 concentration of 2 % where even small variations in the procedure cause considerable changes in the measured ventilation, because of the exponential shape of the N_2 wash-out curve.

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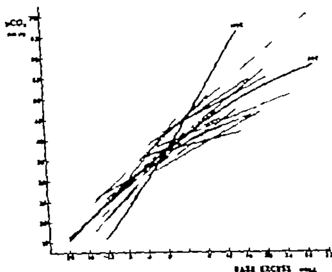


Fig. 2. Evaluation of respiratory compensation in metabolic disturbances of neutrality regulation. Individual regression lines computed for 23 patients recovering from metabolic acidosis or alkalosis. *Heavy lines*: Computed percentiles showing the level to which the arterial pCO must be adjusted to effect 50 % or 100 % reduction of the metabolic pH change (ΔpH_m) corresponding to any actual value of base excess.

department, Odense County and City Hospital, during the last two years as well as 156 observations made in 18 acidotic and recently acidotic infants and children. Most of these suffered from gastroenteritis, diabetes mellitus or nephropathia. Cases of neonatal acidosis are not included.

Values obtained in the presence of pulmonary or cerebral complications are not recorded.

Results

Irrespective of the actual mechanism involved, respiratory compensation must obviously be related in some way to the degree of metabolic disturbance, i.e. to the base excess. In the pCO_2/BE plot of fig. 1 178 corresponding values of BE and pCO representing samples drawn from 20 infants with pyloric stenosis before, during and after treatment as well as 156 similar determinations made in 18 acidotic children are recorded. Excluded from the calculations are the values represented by encircled dots in the figure. These are first observations made on admission in severely acidotic and dehydrated patients. Subsequent measurements gave higher (less negative) values for BE and lower values for pCO_2. On several occasions it was observed that exhausted acidotic children may be temporarily unable to respond to the demands for the physical efforts of pronounced hyperventilation. When rehydration is initiated and the general condition improves compensation again becomes adequate.

The scatter plot of fig. 1 indicates a definite relationship of the capillary pCO to the BE throughout the scale of metabolic disturbance. The regression lines computed for the acidotic and alkalotic groups, respectively are both clearly significant, but the standard deviation

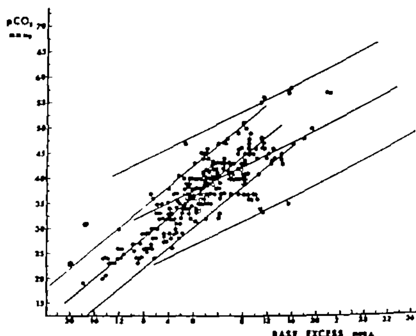


Fig 1 Evaluation of respiratory compensation in metabolic disturbances of neutrality regulation.

Open circles. 178 observations made in 20 infants with pyloric stenosis.

Regression line (method of least squares) pCO = 37 7 + 0.61 BE.

Regression coefficient significantly different from zero $t_{0.001} = 3.3471$ $t > 9$ (176 degrees of freedom).

Standard deviation from the regression line $s_{y \cdot x} = 5\ 46$.

Mean error of th regression coefficient, $s_b = 0\ 064$

Black dots 136 observations mad in 18 acidotic and recently acidotic children.

Regression line pCO = 36 5 + 1 03 BE.

Standard deviation from the regression line, $s_{y \cdot x} = 3.09$.

Mean error of the regression coefficient, $\bar{s}_b = 0.045$

The deviations from the two regression lines are significantly different, $(s^2_{y \cdot x}) / (s^2_{y \cdot x}) = F > 3$, and so are the regression coefficients, $t_{0.001} < 3\ 34$ $t > 5$ (299 degrees of freedom)

Ranges are $\pm 2\ s_{y \cdot x}$.

under which the compensatory mechanism operates and to describe in more quantitative terms the resulting change in pH

Definitions and symbols

The concepts of acid base chemistry are those of the Bronsted theory

BE = base excess" the concentration, in mEq/l of the actual blood volume, of strong base or acid (negative values) corresponding to any actual change in the blood s buffer base, i.e. BE denotes the titratable acid or base of the blood. "Standard bicarbonate = base of the blood. "Standard bicarbonate" = concentration of bicarbonate in plasma after equilibration of the fully oxygenated blood at pCO = 40 mm Hg and temp. = 38 C.

ΔpH_{37} = change in metabolic pH' i.e blood pH at a constant and normal alveolar pCO of 37 mm Hg

BC = "buffer value" (dB/dpH) of van Slyke.

Method and material

The acid base determinations were carried out using the Astrup micro equipment (3, 28, 29) and have been described in more detail elsewhere (19) The pH scale adopted is that of the National Bureau of Standards. Every result recorded is the mean of two or more readings. Oxygen saturations were not measured which means that the pCO values recorded are slightly too low (28)

The case material includes 178 determinations of acid-base status made in 20 infants with pyloric stenosis admitted to the pediatric

in BE. The absolute respiratory change in pH, expressed per mEq of BE, required to secure a given percentage compensation thus decreases with increasing severity of the metabolic alkalosis.

In the initial phases of compensation (fig. 3) the situation is characterized by a comparatively small difference in pCO and a large difference in pH. The partial derivative of the Henderson-Hasselbalch equation $\partial(CO_2)_{total} / \partial pCO = 0.03 (10^{pH-6.1} + 1)$ shows that at high pH values the amount of CO absorbed per mm of pCO increase largely exceeds the amount liberated per mm of pCO decrease at low pH values. As the point of maximum buffer capacity of the hemoglobin system lies about pH 7.30 and as the molar BC of the bicarbonate buffer decreases with rising pH, the BC_{total} of the acidotic and alkalotic bloods will not differ much at the beginning of compensation. Hence, at the start of compensation the change in pH per mm of pCO will increase with rising BE, which corresponds to the flattening of the rectilinear log pCO_2/pH lines with increasing base content of the blood demonstrated by Astrup (4).

As a result of these relationships the percentiles corresponding to the lower degrees of respiratory compensation (including the 50 % percentile) will show decreasing slopes with rising BE.

As the final stage of complete respiratory compensation is approached the situation is reversed, and the initial difference in pH will be replaced by a large difference in pCO and in the total CO concentration. This means that the BC of the blood returning from high pH values loaded with CO will now exceed that of the CO deprived acidotic one. Hence, the absorption of the same amount of CO will induce a smaller pH change in the formerly basic blood and so cause a smaller production of bicarbonate. A smaller rise in the concentration of bicarbonate accompanying the absorption of the same amount of CO_2, however means a larger rise in the concentration of physically dissolved CO_2, i.e. a greater change in pCO_2. Hence, on the alkalotic side, both $\Delta CO_2/\Delta pCO_2$ and $\Delta pH/\Delta pCO$ will decrease with increasing percentage levels of pH compensation, and the 100 % percentile (fig 2) runs a nearly rectilinear course.

Thus the course of the pCO_2/BE compensation line based upon measured clinical values, as shown in figs. 1 and 2, may be considered an expression of the fact that the induced respiratory change stops at the 50 % level of pH compensation, irrespective of the base excess present, — which obviously leaves the question unsettled as to how this adjustment is ultimately realized. Knowledge of this would have to be based upon exact quantitative information of the selective functional dependence of the respiratory center and the chemoreceptive organs upon changes in acidity and in the tensions of the respiratory gases.

Discussion

Without providing direct clues to the physiological details the data presented here prove beyond doubt the existence of respiratory compensation in clinical cases of metabolic alkalosis. The results further seem to emphasize the significance of the pH to the compensatory mechanism.

The advantage to the organism of partial respiratory pH compensation should be recognized. In the case of a complete respiratory normalization of the actual pH the important (and indeed necessary) renal excretion of the base surplus would suffer. The 50 % level

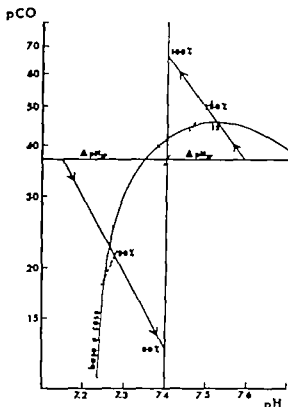


Fig 3 Stepwise representation, in the Siggaard Andersen-Engel nomogram, of the course of the acid-base parameters in respiratory compensation of metabolic alkalosis (BE = + 15 mEq/l) and acidosis (BE = — 15 mEq/l)

Actually of course, compensation sets in gradually as indicated by the broken line (50 % compensation percentile)

For further explanation, see text.

from the regression line is greater in the alkalotic range.

Furthermore, the two regression coefficients differ significantly from each other the average change in pCO per mEq of BE change for the acidotic range being about 1 7 times that found for the alkalotic figures. The implications of this finding will be discussed below

As the level of pCO_2 at which respiratory compensation takes place may be expected to be subject to a normal variation the scatter plot of fig 1 tells nothing about the degree of pCO_2/BE correlation present in the individual patients. In fig 2 are given individual regression lines computed for 23 patients in whom the number of measurements exceeded six. The function relating pCO_2 to BE in respiratory compensation is apparently curvelinear

To estimate the efficiency of respiratory compensation, percentiles were constructed to show for any value of BE, the pCO at which the non-respiratory pH change (ΔpH_{37}) brought about by the relative surplus of non volatile acid or base present would be reduced by some fixed percentage. The percentiles were constructed by means of the log pCO_2/pH lines of the Siggaard Andersen-Engel nomogram corresponding to a hemoglobin concentration of 15 g/100 ml As zero point was used BE + 0 1 pCO_2 37 the means of 24 determinations made in healthy infants aged 1—12 weeks (19)

By trying a number of such compensation percentiles it was found as shown in fig 2 that the 50 % percentile fits the data presented here closely This means that throughout the scale of BE respiratory compensation limits the change in actual pH to roughly one half the corresponding change in pH_{37} and stops here.

It is seen that at the 50 % level of pH compensation achieved by the infants studied here compensation is accomplished by a smaller change in pCO in the alkalotic than in the acidotic range. Each point of a given percentile is determined by the absolute pH change required and by the pH change brought about by a given change in pCO_2. With increasing alkalinity of the blood an increasing fraction of the positive base excess is transported *via* the bicarbonate system (increasing Δ standard bicarbonate/ ΔBE) thus reducing the change in metabolic pH resulting from a given change

subtotal gastrectomy At any rate it seems unlikely that the very slight post prandial alkalosis commonly reported could account for any constant and measurable elevation of the arterial pCO and Dodds did not report on metabolic acid-base changes accompanying the meals. Moller (22) was unable to detect any rise in the arterial pH or standard bicarbonate following Ewald s test meal.

Perhaps more relevant to the present discussion is the observation by Semple and Jones (26) of normal (or slightly in creased) pCO values in 4 patients with hypokalemic alkalosis. The interesting concept of combined intracellular acidosis and extracellular alkalosis in acute ex perimental hypokalemia (7 8, 9) has been put forward as a possible explanation of this relationship but its validity in the chronic hypokalemic alkalosis of clinical cases remains to be proved, and at any rate one would expect low and not normal pCO values in intracellular acidosis. Furthermore, the observation of low pO values in the patients of Semple and Jones may possibly indicate that the pCO values are erroneously low Possibly in some types of metabolic alkalosis the distribution of the hydrogen ion deficit between the extracellular and intracellular compartments may be modified by altered membrane potentials. In most of the patients of the present study potassium deficiency was indicated by low serum levels. The pCO values suggest increased intracellular alkalinity but in the urine a positive net acid was almost constantly excreted, probably because of the concomitant potassium and sodium depletion The raised arterial pCO does probably not per se stimulate the excessive production of an acid urine. At any rate, low pCO does not appear to have any significant inhibitory effect upon the excretion of surplus hydrogen ion in metabolic acidosis.

It is interesting to note that in most of the cases in which a raised arterial pCO has been found accompanying metabolic alkalosis (2, 16 21 22 and the present study) the latter has resulted from vomiting, whereas the patients observed by Semple and Jones lost hydrogen ion through the kidneys. Potassium deficiency may of course be found in both types of alkalosis.

Decreased muscular tone was not a conspicuous feature of the cases studied here and a fairly rapid decrease in the pCO in response to NH therapy was often observed. Thus, hypokalemic weakness of the muscles of respiration could hardly have caused the observed elevation of the pCO values. The respiratory compensation was equally well pronounced in pre- and postoperative alkalosis.

In metabolic alkalosis the induced hypercapnia is probably the primary limiting factor in the respiratory compensation. A rising pCO stimulates respiratory activity and indirectly by depressing the pH weakens the stimulus of compensation. It should be noted that the highest pCO values recorded during the present study (about 70 mm Hg) correspond to the level of maximum respiratory stimulation (5) It has been suggested (10 15, 24) that hypoxemia resulting from the hypoventilation represents another limiting factor and this may be so However to the knowledge of the author the respiratory effects of subacute or chronic oxygen lack in the presence of hypercapnia are not known according to the 'multifactor theory of Gray (15) these stimuli are additive. Measurements of the pO were not under taken in the study presented here.

of respiratory compensation involves a certain protection against large pH changes, at the same time permitting the renal conservation of H^+ to proceed

The questions of why and how respiratory compensation in metabolic alkalosis has so long escaped general notice may possibly lead to the recognition of different biochemical patterns of alkalosis The available evidence is meager In 1920 Davies et al (11) demonstrated in auto-experiments, a small transient increase in the alveolar CO percentage following the ingestion of 30—57 g of bicarbonate. A maximum increase of about 1 vol % corresponding to a rise in pCO_2 of 7—8 mm Hg was reached 3—4 hours after the test dose was taken. These results were advanced by Peters and van Slyke (23) in support of the concept of respiratory compensation in metabolic alkalosis However the bicarbonate was always taken "shortly after a massive breakfast and in 1921 Dodds (12) found a postprandial rise in the arterial pCO of the same order of magnitude following meals containing no bicarbonate. The liberation of CO_2 in the alimentary canal experienced by one of the authors may have introduced another error Furthermore, it has been suggested (18) by essentially *in vitro* argument, that the liberation of CO_2 in the blood resulting from the engagement of administered bicarbonate with the non volatile blood buffers might cause an increase in the arterial pCO_2 but this could hardly be so

If in the course of 3 hours the BE has risen to + 10 mEq/l the standard bicarbonate will show an increase of about 8 mEq/l. Disregarding the difference between whole blood and plasma bicarbonate concentrations this means that during this period about 2 mEq of CO_2 have been released corresponding to the increase by this amount of the non-volatile buffer base. If, in the meantime, the pCO_2 has increased a little the production of CO_2 will be still less. In an adult individual (blood volume 5 liters, total energy expenditure 3 000 cal./24 h RQ 0.82) each liter of blood will in 3 hours carry as much as 5—600 mEq of CO_2 to the lungs! If the BE of + 10 mEq/l has resulted from a primary loss of hydrogen ion (vomiting, renal H^+ loss) the corresponding absorption of CO_2 will be about 8 mEq/l — or less if the pCO_2 is to fall.

The level of pCO_2 is certainly not directly influenced by such small changes in the net load of CO_2 requiring pulmonary excretion but is solely determined by the factors governing physiological regulation of breathing These considerations are quite consistent with earlier observations (13 25) that only following rapid intravenous injection does bicarbonate elicit a slight increase and sodium carbonate (and hydroxide) a small transient decrease in the pCO_2 whereas slow injections of these substances do not disturb the ventilation appreciably Such slight initial respiratory changes (i.e. excretion or retention of extra CO_2) during accumulation of volatile and nonvolatile base represent physiological adjustments maintaining a constant and normal arterial pCO rather than a compensatory response to an altered acid-base status. They probably indicate that extracellular pCO changes are more rapidly reflected inside the cells than is a raised extracellular pH

The findings of Dodds (12) have been cited (31) as an example of respiratory compensation in metabolic alkalosis. One may wonder if diaphragmatic inhibition by the filled stomach has not played a significant role which would be in keeping with the observation by Dodds that no postprandial rise in pCO occurred in a person who had previously undergone

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Cerebral Complications in Nitroglycerine Treatment of Angina Pectoris

By

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By a direct influence on the peripheral vessels, nitroglycerine produces a generalised vasodilatation, especially of the capillaries, ... ules and veins (16 17) In experimental studies on the blood flow it has been shown that nitroglycerine dilates the coronary vessels of animals (7) and the same observation has been mad in healthy human subjects (1 6) It is generally assumed that the effect of nitroglycerine in angina pectoris is due to this dilatation of the coronary vessels This concept was challenged by Gorlin et al. (4) who claimed that, on the contrary nitroglycerine reduces the blood flow of the coronary vessels in patients with angina pectoris. However the most recent study within this field (6) lends strong support to the classic concept these authors demonstrated a significant increase in the myocardial blood flow during nitroglycerine treatment, also in patients with coronary sclerosis.

For nearly a century nitroglycerine has been the most useful drug in the treatment of stenocardial attacks. However it may fail in typical angina pectoris, and it may even provoke attacks (2) In a study of 158 patients with a pathological electrocardiographic response to exercise, Russek et al. (12) found that a dose of 0.4 mg nitroglycerine increased the ischaemic changes in about 10 % of the cases, and that larger doses accentuated the pathological changes in some additional cases. The authors offered the explanation that the effect on the systemic circulation — accumulation of the blood in the veins of the lower limbs, diminished venous return and a reduction in the cardiac output with resultant tachycardia and hypotension — is so considerable that coronary circulation is reduced in spite of dilatation of the coronary vessels.

Nitroglycerine may occasionally give rise to appreciable blood-pressure falls, especially in the standing position (4 9 10, 12) It is therefore not surprising that vascular collapse or even coronary occlusion provoked by nitroglycerine in thera

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Conclusions and summary

The presence of respiratory compensation in alkalotic infants with pyloric stenosis as well as in acidotic patients is clearly demonstrated

The chemical relationships conditioning the influence of respiratory changes on blood pH in acidosis and alkalosis are discussed on the basis of the Siggaard Andersen Engel nomogram

On the basis of measured clinical values it is shown that throughout the scale of metabolic disturbance of neutrality regulation respiratory compensation limits the change in actual blood pH to approximately one half the corresponding change in metabolic pH

Addendum

Recalculation of the data according to the revised base excess curve, recently published by Siggaard Andersen (Scand. J clin. Lab. Invest. *14* 598, 1962) causes a slight leftward displacement and counter-clockwise rotation of the system of two regression lines shown in fig 1 The 50 % percentile is displaced correspondingly This correction does not influence any of the statements and conclusions contained in the above article.

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right side. There was no paresis of the lower limbs but both plantar responses were extensor. The patient complained of dizziness, and mild expressive aphasia was noticed. The paresis of the facial nerve and right arm subsided within a few days, and at discharge fortnight later the patient felt well, speech was normal, and the dizziness had disappeared.

Electrocardiography showed left-sided axis deviation, blood pressure 170/100—150/105. Ophthalmoscopy revealed slight vascular nickings, but otherwise normal conditions.

Discussion

It is well known that a sudden fall in blood pressure may produce cerebral infarction in elderly persons (3) and Shanbrom and Levy (13) established that even a modest reduction in blood pressure may produce or aggravate focal neurological symptoms in patients with occlusive disease of the carotid or basilar artery.

In the three patients considered in the present paper symptoms of cerebral ischaemia developed after they in the standing position, had taken nitroglycerine sublingually in therapeutic doses (0.5—1.0 mg). It is likely that the symptoms were provoked by the hypotensive effect of the drug. However, we do not know the blood-pressure levels at the time of the insults, and obviously it cannot be excluded that the occurrence of cerebral symptoms immediately after the ingestion of nitroglycerine may have been quite accidental. However, in the first two cases the causal relationship seems to be fairly convincing. The first patient, who had never fainted before, experienced two syncopal attacks at an interval of a few days, on both occasions a few minutes after the ingestion of nitroglycerine. In the second patient, who rarely used nitroglycerine, the cerebral symptoms occurred a few minutes after he had, in the standing position taken twice his usual dose of nitroglycerine in order to ensure a good effect. It is likely that a causal relationship existed in the third case, but there is some uncertainty as to the ingestion of nitroglycerine because the patient did not clearly remember the time immediately before the insult.

In case 1 the patient had never before had cerebral symptoms, and the two uncomplicated syncopal attacks did not entail serious consequences. In cases 2 and 3 signs of cerebral arteriosclerosis had previously been present. The second patient had had transitory paresis of the same localisation 3 years previously and the third patient suffered from impaired memory and slight mental deterioration. It is therefore likely that these two patients suffered from pre-existing relative cerebral circulatory insufficiency which after the orthostatic blood-pressure fall induced by nitroglycerine gave rise to focal cerebral symptoms.

Considering that patients who receive nitroglycerine are often elderly persons with appreciable generalised arteriosclerosis it is surprising that cases of actual cerebral complications during this therapy have not previously been reported. As appears from case 3 it must be assumed that this is partially due to the fact that it is often difficult for the patients afterwards to recall the happenings immediately before the insult. Another reason may be that it is only during recent years that special attention has been focused on the significance of extracerebral factors in the development of cerebral infarction (5, 11 with further references).

As already pointed out, I have been unable to trace previous reports on actual

peutic doses (0 3—1 3 mg) has been observed (8 9 14)

As far as I have been able to trace cases of cerebral complications during nitroglycerine treatment have not previously been described in the literature. In this clinic, we have during the last few months seen three patients with cerebral symptoms occurring in association with administration of nitroglycerine.

Case reports

Case 1 A woman aged 74 with a past history of good health, had for about 12 months suffered from dyspnoea on exertion, and for the last 2 months exertion had often resulted in precordial pain radiating to both arms and the back. Sublingual administration of nitroglycerine gave prompt and effective relief of the pain. During a walk, the patient had an attack of the usual pain and took the usual dose of nitroglycerine. A few minutes later she felt weak and dizzy and fell. She did not know whether she had been unconscious. She was taken to the accident ward and admitted to hospital. On admission, she felt well, but tired. No cerebral symptoms were observed. The patient stated that a few days previously she had had a similar fainting fit after having taken nitroglycerine. She had never previously suffered from syncope. The patient was discharged a few days later

Electrocardiography (limb and precordial leads, V_{1-6}) showed no abnormalities except a few extrasystoles. Blood pressure 150/85—180/90

Case 2 The patient was a man aged 61 who had been in good health until symptoms of coronary occlusion developed 9 years ago. Since then, he had on rare occasions (less than once monthly) had attacks of thoracic oppression and slight dyspnoea. Sublingual administration of nitroglycerine, 0.5 mg usually resulted in prompt relief Three years ago, he had had an attack of left-sided hemiparesis lasting 3 hours t occurred without known provocation and disappeared completely

On the day of admission, he had one of his usual attacks. As he was busy he took, contrary to his usual practice, two tablets, i. e. 1 0 mg nitroglycerine. A few minutes later he became so dizzy that he had to sit down at once. When he tried to get to his feet again, he fainted and fell in the street, and was then taken to hospital.

On admission, he was conscious and rational, without disturbances of speech, but physical examination revealed left-sided facial palsy of central type. The muscular power was reduced in the entire left arm, but normal and equal in both legs. The tendon reflexes were equal plantar reflexes of the extensive type were elicited. The next day the plantar reflexes were normal. There was pronounced dysdiadochokinesia of the left arm but the muscular power was normal. Within a week, muscular co-ordination and diadochokinesia returned to normal. The facial palsy also subsided so that only a slight impairment of the mimic function of the area supplied by the left facial nerve remained.

Electrocardiography showed signs of ischaemic cardiac disease blood pressure 130/70—125/80 Ophthalmoscopy revealed unequally calibrated arteries and moderate vascular nickings.

Case 3. A man aged 61 had for about 2 years suffered from intermittent claudication due to occlusion of the left and right femoral arteries (confirmed by arteriography). During the last 2 years he had suffered from typical angina pectoris, on which good effect had been obtained by sublingual administration of nitroglycerine tablets (dose 0.5 mg) According to the family doctor slight mental deterioration had been present during the last year During the last few months, he had experienced repeated attacks of slight, uncharacteristic dizziness.

Two days before admission, the patient had a mild right-sided paresis and fell in his office The patient stated that he had probably taken a nitroglycerine tablet just before this occurred but he was not quite sure as it was difficult for him to recall the happenings during the last few minutes before the insult.

On admission, physical examination revealed right sided facial palsy of central type reduced muscular power of the right arm and slightly exaggerated tendon reflexes on the

Three cases in which complications occurred during the treatment with nitroglycerine are reported. The doses taken were 0.5 1 0 and 0.5 mg respectively.

In the first case, the patient collapsed twice with an interval of a few days immediately after ingestion of nitroglycerine in the standing position. In cases 2 and 3 ingestion of nitroglycerine in the standing position was followed by symptoms of cerebral infarction, probably because these two patients were sensitive to reduction in blood pressure on account of a pre-existing relative circulatory insufficiency. The causal relationship between the intake of nitroglycerine and the cerebral ischaemic symptoms seemed to be convincing in the first two cases, while it was less certain in the third case, because this patient did not clearly remember if he had taken nitroglycerine just before the insult.

It is concluded that nitroglycerine therapy is not without hazards, since complications may occur even when normal therapeutic doses of 0.5—1.0 mg are used. It is therefore advised to prescribe an initial dose of 0.25 mg. This is in agreement with previously reported clinical and experimental experience showing that this is the optimum dosage level in most cases. The dose should not be increased unless it proves to be ineffective. In addition, it is recommended that the patients should be instructed never to take the drug in the standing position. If these precautions are observed, it will presumably be possible to avoid complications.

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cerebral complications in this therapy but in two older publications attempts were made to analyse the incidence of collapse occurring in relation to treatment with nitroglycerine. Prodger and Ayman (9) who used relatively large doses (0 65—1 3 mg) encountered collapse in four out of 110 cases. The blood pressure was unobtainable for some time in two of the cases and fatal coronary occlusion developed in one of these. Sprague and White (14) used a lower dosage and reported three cases of collapse among 900 patients two occurred after a dose of 0 65 mg and the third after 0 3 mg nitroglycerine. In one of these three patients coronary occlusion developed As already pointed out, Russek et al (12) reported that nitroglycerine may aggravate the ischaemic changes in patients with abnormal electrocardiographic response to exercise Like Prodger and Ayman and Sprague and White, they therefore recommended an initial dose of 0.2—0 3 mg and found that this is the optimum dosage level in most cases

It is undoubtedly due to mere chance that complications were seen in three cases within the space of 3—4 months in the same clinic, but viewed in connexion with the above mentioned analyses of the collapse frequency in nitroglycerine therapy these cases nevertheless show that the use of this agent may be dangerous. It must therefore be considered what can be done to minimise the inherent risk.

It should in fact, be relatively easy to prevent these complications. Above all the initial dose should be small e. g 0 25 mg and only if it proves ineffective should this dose be increased Furthermore symptoms of cerebral arteriosclerosis should call for special care in the fixation of the dosage level Similar considerations presumably apply to hypertension since Weiss and Ellis (15) and Lueth and Hanks (6) observed that blood pressure diminutions and syncope are apt to develop in hypertensive patients after administration of nitroglycerine or the closely related sodium nitrite. The risk involved is greatest in the standing position. The blood pressure fall after ingestion of nitroglycerine may result in dizziness and malaise, which can be counteracted simply by lying down (12) Patients with such symptoms should be instructed never to take nitroglycerine tablets in the standing position. Finally all patients should be told that if one or two doses do not give relief of a severe attack of angina pectoris, they must not take more nitroglycerine, but should instead call the doctor Patients in this situation will sometimes take large doses of nitroglycerine. This involves the risk of a considerable fall in blood pressure which may be dangerous whether the pain is due to a severe attack of angina pectoris or to incipient coronary occlusion.

Nitroglycerine is rightly considered to be an excellent agent for the relief of stenocardial attacks, but it must be borne in mind that it has a strong circulatory effect which may sometimes bring about a considerable reduction in the blood pressure. The purpose of the present paper is to call attention to the risk involved even after administration of what is normally considered to be therapeutic doses.

Conclusion and summary

A brief survey is given of the action of nitroglycerine on the vascular system, with special reference to its hypotensive effect.

Three cases in which complications occurred during the treatment with nitroglycerine are reported. The doses taken were 0.5 1.0 and 0.5 mg, respectively.

In the first case, the patient collapsed twice with an interval of a few days immediately after ingestion of nitroglycerine in the standing position. In cases 2 and 3 ingestion of nitroglycerine in the standing position was followed by symptoms of cerebral infarction probably because these two patients were sensitive to reduction in blood pressure on account of a pre-existing relative circulatory insufficiency The causal relationship between the intake of nitroglycerine and the cerebral ischaemic symptoms seemed to be convincing in the first two cases, while it was less certain in the third case, because this patient did not clearly remember if he had taken nitroglycerine just before the insult.

It is concluded that nitroglycerine therapy is not without hazards since complications may occur even when normal therapeutic doses of 0.5—1.0 mg are used. It is therefore advised to prescribe an initial dose of 0.25 mg. This is in agreement with previously reported clinical and experimental experience showing that this is the optimum dosage level in most cases. The dose should not be increased unless it proves to be ineffective. In addition it is recommended that the patients should be instructed never to take the drug in the standing position. If these precautions are observed it will presumably be possible to avoid complications.

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Hypoalbuminaemia in Ulcerative Colitis and Certain Forms of Enteritis

Clinical and Pathophysiological Aspects

By

J Wetterfors, S.-O Liljedahl, L.-O Plantin and G Birke

In intestinal disorders of inflammatory origin, hypoproteinaemia, and especially hypoalbuminaemia, is a common feature. In ulcerative colitis and regional enteritis the changes in serum-proteins have been extensively studied (1 2, 4 7 8, 9 23 24)

The relationship of this serum-protein deficiency to the disease and its mechanism have been discussed. Hepatic insufficiency of varying degree, with or without cirrhosis has been observed in ulcerative colitis and held responsible for the hypoproteinaemia/hypoalbuminaemia, i.e. an impaired synthesis (15 19 20 28) But hypoalbuminaemia has also been observed as a transitory phenomenon following relapses and remissions in cases where no impairment of the liver function has been demonstrated (23 24)

Malabsorption has been thought to play a role in the albumin deficiency especially in extensive regional enteritis (9) Malnutrition as a causative factor possibly via insufficiency of the liver has been proposed (4 15 19)

Welch et al. (29) demonstrated an increased faecal output of nitrogen, often 5—6 times the normal, in patients with severe ulcerative colitis. This high nitrogen content was believed to depend on losses of protein partly in the form of plasma and exudate into th diseased bowel. To some extent this opinion was supported by Turnen et al. (28)

By using ^{131}I-albumin, Steinfeld et al. (25) in 1957 demonstrated an increased faecal output of radioactivity in ulcerative colitis and regional enteritis indicating a pathological leakage of albumin through the diseased bowel wall. More detailed studies on this subject were published later (3, 26 30)

The purpose of this investigation is not only to give further evidence of the leakage of albumin, but also to correlate the

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leakage and the degradation of albumin to the clinical picture and the duration of the disease, as well as to throw new light on the behaviour of blood and plasma volumes on the intravascular albumin and on the distribution of albumin Another aspect, not discussed earlier is the albumin situation post operatively i.e. after total colectomy

Material

Ulcerative colitis

Seventeen patients with ulcerative colitis were investigated (tables I and IV) Six were women and 11 men, whose ages ranged from 17 to 58 years. The diagnosis was established by radiographic examination and rectoscopy In 8 cases the diagnosis was further verified at operation.

All the patients had on many occasions had a bloody discharge, haemorrhages from the large bowel, or bloody stools. During the investigation by means of ^{131}I-albumin macroscopically bloody stools or a strong guaic reaction were occasionally present in only 4 patients (cases 5 6 8 and 13) Actual body weights and heights are recorded in table I. Since it was in most cases impossible to obtain accurate values for the true weight losses, these are not tabulated. It will be seen in table I that in most cases the entire colon and rectum were involved in the pathological process.

Six patients (cases 1—6) had acute but not fulminant relapses of their disease while under investigation. All had frequent diarrhoeas (except case 4) and a more or less impaired general condition Thus the word acute does not refer here to the fulminant form of colitis with an acute onset. The duration of colitis was 1—11 years. One of the patients (case 2) was investigated twice with an interval of 6 months. In case 1 with an 11 year history of colitis there was malignant degeneration. Certain clinical data are set out in table I

Seven patients (cases 7—13) were, when investigated, in a more quiescent but not remittent phase of the disease. They are classified as non-acute cases. Patients nos. 7 and 8 were just recovering from acute relapses. All had diarrhoeas. The duration of the disease in these patients varied between 4 months and 14 years. Patient no. 11 had perianal and periproctitic abscesses, which were incised immediately before and during the investigation. In case 12 colectomy with ileoproctostomy had been performed 10 months earlier In case 10 there was also malignant degeneration (see table I)

In 3 patients (cases 14—16) studies were performed in remission. Their body-weights were still below normal, but they were in a good general condition and all except case 15 had solid stools (table I)

Three patients were studied 6—27 months after total colectomy with permanent ileostomy (cases 4 11 and 17 the latter was not investigated preoperatively) They were all in excellent health and had gained 11—15 kg in weight (table IV)

Enteritis

One patient with subacute jejunoileitis o unknown aetiology of 3 months duration (case 18) and six patients with Crohn's disease (cases 19—24) were also investigated. Their ages ranged from 19 to 69 years. Clinical data on these patients are set out in table VI. A positive guaic reaction, but no macroscopically bloody stools was occasionally found in case 18. Moderate anaemia was present in cases 20 21 and 24 and albuminuria in case 18 (∾1 /‰)

Patient no. 18 had frequent diarrhoeas throughout the period of investigation. Six months after this study he had fully recovered. The plasma proteins were by then normal

Cases 19 and 20 were relatively acute. In the former the process was localized to the terminal ileum and the entire colon including the rectum. This patient was investigated twice, the first time when the ileum was divided and a permanent and a wet ileostomy established. The second investigation was made 6 months after total colectomy and resection of th diseased part of the ileum. Histological examination showed changes as in Crohn's disease.

Patient no. 20 with terminal ileitis died on the 5th day of the study from pulmonary embolism. Thus only data on the distribution phase were obtained.

Case 21 (see table VI) was investigated twice with an interval of 1 year

Table I. Clinical data on the patients with ulcerative colitis

Cases 1—6 acute. Cases 7—13: non-acute. Cases 14—16 in remission.

Case no., Sex	Weight (kg)	Height (cm)	Age (yrs)	Duration of disease (yrs)	Radiographic extension of disease	Daily frequency of diarrhoea	Guaiac reaction of faeces	General clinical picture of patient during investigation
1 ♂	62	183	29	11	Entire colon (+ cancer of sigmoid flexure)	8	(+)	Bad general condition for 1 month. Now slight improvement general weakness no weight-gain; anaemia. Operated later
2 ♂	64 68	178	56 57	2—3	Entire colon	20—30	(+)	Acute relapse for 1 month. Slight improvement before but deterioration during investigation; general condition poor; slight weight-loss; slight anaemia
3 ♂	71	178	58	2	Transverse colon-rectum	10	(+)	Acute relapse for 3 weeks. General condition poor slight weight-loss; anaemia
4 ♀	51	161	57	8	Entire colon	4	(+)	Acute relapse for 1.5 months. Steroid therapy led to improvement. Just before and during investigation impairment of general condition without increase of diarrhoea. Mucosal gangrene, partial dilatation and gross oedema of bowel at operation
5 ♀	41	163	17	3	Entire colon	7	+	Acute deterioration in connection with "cold". General condition slightly worsened. Weight-loss insignificant; anaemia. Operated later
6 ♂	50	173	28	1	Entire colon	10—15	+++	Acute phase with poor general condition; ACTH therapy weight-gain 1–2 kg; fever anaemia. Operated later
7 ♂	52	170	22	4	Entire colon + terminal ileum	4	(+)	Onset of acute relapse 1 month earlier. Quiescent during investigation; general condition good; weight constant anaemia. Oper

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Case 21 (see table VI) was investigated twice with an interval of 1 year

Patients nos. 22 and 23 with clinical and radiographical recurrences in the terminal preileum at the ileotransversostomies after previous ileo-coecal resections both had continuous diarrhoea, but were in a good general condition.

Patient no. 24 had terminal ileitis involving part of the ascending colon. She was investigated in remission but had perityphlitic abscess, which was incised during the investigation.

Fifteen control cases were studied. A report on these has been published earlier (31)

Transfusion of albumin

In case 6 44 g of albumin were given the second week of investigation and in case 19 the same amount on days 10—14. In the rest no or insignificant amounts of albumin were given.

Methods

^{131} I-albumin from Radiochemical Centre Amersham, England, (10—1 μC/mg albumin) was used in the 7 patients first investigated (cases 1 9 11, 12, 14, 15, 19) In all other cases RIHSA-M from Abbott and ^{131}I-albumin prepared t King Gustaf V's Research Institute were used (5 μC/mg albumin) The latter was prepared by a modified McFarlane technique.

Dialysis and electrophoresis for control of free ^{131}I-activity and purity were performed in the usual way

The doses given varied between 25 and 45 μC.

The samples of whole blood, plasma and urine were treated as described in previous paper (31) A sufficient number of counts to ensure an accuracy of t least ± 2 % was registered. The daily faecal collections were treated as follows. During the first part of the investigation the non-solid samples were measured directly in special tins, the solid ones after slight homogenisation in water The amount of radioactivity was calculated by comparison with known standard measured in the same way The accuracy was ± 10 %. I the 14 cases latterly investigated complete homogenisation of the samples was performed and the activity measured on 2-ml samples with an accuracy of ± 5 %

Thyroid blocking was performed in the usual manner with Lugol's solution. That no activity had accumulated in the thyroid was ensured by external counting (31) Only in cases 15 and 19 (first study) where the blocking was insufficient, high activities were registered (15—30 %)

Blood, plasma and red-cell volumes (TBV PV and RCV) were determined by methods fully described elsewhere (31) On comparing these volumes between the different groups and the controls, the values were also referred to height and body-weight.

Serum proteins

Total protein was determined by Kjeldahl' method, and the different protein fractions by paper electrophoresis in veronal buffer (18) These determinations were made with intervals of 3 to 7 days.

Normal values for total protein are 6.5—8.0 g/100 ml and for albumin 4.0—5.5 g/100 ml (27)

Intravascular albumin was calculated from the albumin concentration and the plasma volume. The same parameters as for blood volumes were used when comparing mean values between different groups.

Breakdown and losses of albumin

The degradation (catabolism) of albumin, i.e. true catabolism plus losses, was estimated by the method of Campbell et al. (5) which is valid when degradation is intravascular That is, it was expressed as per centage of the intravascular albumin-pool:

$$\frac{\text{Activity in urine and faeces per 24 h.}}{\text{Mean activity in plasma for corresp. 24 h.}}$$

For cases in which there was a steady state as far as distribution was concerned, determinations by Matthews' (16) method gave concordant values. As this method is not valid in cases with delayed or non-steady state, the figures obtained, which are too high, will not be presented. This divergence between the two methods is due to the fact that the fall of the plasma activity curve expresses not only the catabolism but also the pathological distribution (31)

Values for total catabolism (including faecal losses) as well as for "true" catabolism (solely urinary activity) wer calculated.

Table I (cont.)

Case no., Sex	Weight (kg)	Height (cm)	Age (yrs)	Duration of disease (yrs)	Radiographic extension of disease	Daily frequency of diarrhoeas	Guaiac reaction of faeces	General clinical picture of patient during investigation
8 ♂	62	175	51	$\frac{1}{2}$	Entire colon + terminal ileum	4	+++	Quiescent phase after acute onset 4 months earlier. General condition good weight constant no anaemia
9 ♂	53	166	28	10	Descending colon rectum	10	−	Good general condition weight-gain constant diarrhoeas no anaemia
10 ♂	60	176	26	13	Entire colon (+ multiple cancer of colon)	8	(+)	1 month before investigation slight increase of diarrhoeas. General condition good slight anaemia. Operated later
11 ♂	61	176	30	14	Entire colon-rectum	4	−	Good general condition weight-gain; anaemia. Operated later
12 ♂	54	162	37	1−2	Ileo-proctostomy Rectum diseased	10	−	General condition fairly good, improving weight constant no anaemia
13 ♀	48	158	23	9	Half transverse colon	5	+++	General condition good. Picture influenced by psychogenic components weight constant no naemia
14 ♀	44	156	18	3	Entire colon	0	++	Good general condition weight-gain, slight anaemia. Operated later
15 ♀	35	153	22	4	Ascending colon	4−6	−	Good general condition; Rel pylorostenosis. Weight constant slight anaemia
16 ♀	45	158	17	1	Entire colon	3	−	Fairly acute 3 months before investigation. ACTH-therapy led to continuous improvement. General condition during investigation good weight-gain no anaemia

Patients nos. 22 and 23 with clinical and radiographical recurrences in the terminal ileum at the ileotransversostomies after previous ileo-coecal resections both had continuous diarrhoea, but were in a good general condition.

Patient no. 24 had a terminal ileitis involving part of the ascending colon. She was investigated in remission but had a perityphlitic abscess, which was incised during the investigation.

Fifteen control cases were studied. A report on these has been published earlier (31)

Transfusion of albumin

In case 6 44 g of albumin were given the second week of investigation and in case 19 the same amount on days 10—14. In the rest no or insignificant amounts of albumin were given.

Methods

[131] I-albumin from Radiochemical Centre Amersham, England, (10—15 μC/mg albumin) was used in the 7 patients first investigated (cases 1 9 11 12, 14 15, 19) In all other cases RIHSA M from Abbott and [131]I-albumin prepared at King Gustaf V Research Institute were used (5 μC/mg albumin) The latter was prepared by a modified McFarlane technique.

Dialysis and electrophoresis for control of free [131]I-activity and purity were performed in the usual way

The doses given varied between 25 and 45 μC.

The samples of whole blood, plasma and urine were treated as described in previous paper (31) A sufficient number of counts to ensure an accuracy of at least ± 2 % was registered. The daily faecal collections were treated as follow During the first part of the investigation the non-solid samples were measured directly in special tins, the solid ones after slight homogenisation in water The amount of radioactivity was calculated by comparison with a known standard measured in the same way The accuracy was ± 10

In the 14 cases latterly investigated complete homogenisation of the samples was performed and the activity measured on 2-ml samples with an accuracy of ± 5

Thyroid blocking was performed in the usual manner with Lugol' solution. That no activity had accumulated in the thyroid was ensured by external counting (31) Only in cases 15 and 19 (first study) where the blocking was insufficient, high activities were registered (15—30 %)

Blood, plasma and red-cell volumes (TBV PV and RCV) were determined by methods fully described elsewhere (31) On comparing these volumes between the different groups and the controls, the values were also referred to height and body-weight.

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Total protein was determined by Kjeldahl's method, and the different protein fractions by paper electrophoresis in veronal buffer (18) These determinations were made with intervals of 3 to 7 days.

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$$\frac{\text{Activity in urine and faeces per 24 h.}}{\text{Mean activity in plasma for corresp. 24 h.}}$$

For cases in which there was a steady state as far as distribution was concerned, determinations by Matthews' (16) method gave concordant values. As this method is not valid in cases with delayed or non-steady state, the figures obtained, which are too high, will not be presented. This divergence between the two methods is due to the fact that the fall of the plasma activity curve expresses not only the catabolism but also the pathological distribution (31).

Values for total catabolism (including faecal losses) as well as for "true catabolism (solely urinary activity) were calculated.

Table II. Total blood volume (TBV), plasma volume (PV) and intravascular albumin (Alb) totally and referred to height (l/m, g/m) and body-weight (ml/kg, g/kg) in the different groups of ulcerative colitis cases and controls (Mean ± S. D.)

Cases 1—6: acute. Cases 7—13: non-acute. Cases 14—16: in remission.

Cases	TBV	TBV/m	TBV/kg	PV	PV/m	PV/kg	Alb.	Alb./m	Alb./kg
1—6	4.46 ± 0.94	2.55 ± 0.45	76.9 ± 11.8	3.14 ± 0.71	1.80 ± 0.35	54.2 ± 9.8	93.7 ± 22.1	53.7 ± 10.8	1.61 ± 0.19
7—13	4.60 ± 0.79	2.71 ± 0.34	82.3 ± 7.5	3.17 ± 0.55	1.86 ± 0.27	56.7 ± 7.7	113.3 ± 17.3	66.8 ± 8.3	2.03 ± 0.19
14—16	2.74 — 3.51	1.79 — 2.22	72.0 78.0	1.86 — 2.70	1.22 — 1.71	51.8 — 60.0	69 — 105	45.1 — 66.5	1.82 — 2.33
Controls									
n = 15	4.74 ± 0.80	2.79 ± 0.37	72.0 ± 8.88	3.10 ± 0.45	1.82 ± 0.21	47.2 ± 5.97	127.9 ± 21.3	73.2 ± 10.3	1.95 ± 0.31

Distribution of albumin

The relation between the slopes of the curves describing intravascular and total retained activity has been pointed out earlier (31). The quotient between the elimination constants for intravascular and total retained activity (Kiv/Krt) is a numerical but relative index of pathological distribution. This ratio was determined, except in cases 9 and 15 in which the collections of excretions were not complete. In those cases in which the quotient Kiv/Krt was within normal range the extravascular pools of albumin were determined.

Protein-bound activity in faeces and faecal excretion of ^{131}I-albumin and ^{131}I iodide

Faecal specimens from 2 patients (cases 6 and 8) were homogenised in physiological sodium-chloride. After centrifugation and filtration, carrier albumin was added. Precipitation with 20 % trichloracetic acid and, on the supernatant, with 5 % phosphotungstic acid was performed. Precipitation with silver nitrate was then done after addition of potassium iodide. The radioactivity of the different precipitates was measured.

In case 7 the faecal excretion of ^{131}I iodide administered intravenously was investigated about 1 month after the albumin study. The patient's clinical condition was unchanged between the two investigations.

Intestinal and gastric juices

In case 18 with jejuno-ileitis jejunal and gastric juice was aspirated, and the amount of protein-bound activity estimated. Electrophoresis was also performed on the intestinal and the gastric juice.

Other investigations

Liver function was studied by means of the thymol turbidity test, bilirubin, prothrombin index and alkaline phosphatases in all cases excluding 5. In a few patients (cases 7, 8 and 14) bromsulphthalein or prontosil-tests were performed as well. In one (case 19) needle biopsy of the liver was made.

Tests for malabsorption (vitamin B_{12}, vitamin A and xylose tests) were made in 4 patients (cases 8, 12, 15 and 20).

To obtain a rough estimate of the proportion of albumin nitrogen in the total amount of faecal nitrogen, the latter was determined by Kjeldahl's method in case 10, simultaneously with the ^{131}I-albumin study.

Statistical methods

Mean (M) and standard deviation (S.D.) were determined in the usual way and the levels of significance with t test for small sam-

Table III Data on the cases with ulcerative colitis Intra- and extra-vascular albumin pools (I.v. and E.v., respectively) r.a. = radioactivity numbers in parentheses denote days of collection, when no number is given, this period is 11 days. Am = [illegible] I-albumin Amersham

Case	Alb. conc. (g/100 ml) initially and at end of invest.	I.v. alb.	E.v. alb.	Faecal loss of r.a. in % of dose	Total degradation of albumin: % of I.v. pool/d	g/d	g/d/kg	True degradation (excluding faecal losses): % of I.v. pool/d	g/d	g/d/kg	Calculated synthesis of albumin	
1	3.0–2.7	118	141	8.4 (7)	26	30.7	0.49	21.1	24.9	0.40	—	Am
2	2.8–1.9	84	—	12.4	—	—	—	—	—	—	—	
	2.9–3.2	109	134	32.5	17.9	19.5	0.29	6.7	7.3	0.11	Increased	
3	3.8–2.4	118	132	25.1	12.7	15.0	0.21	6.2	7.3	0.10	Normal	
4	2.9–3.4	81	—	3.8 (6)	10.7	8.7	0.17	7.6	6.1	0.12	Normal	
5	3.2 2.9	59	72	23.9	16.8	9.9	0.24	10.0	5.9	0.14	Normal	
6	2.5–2.6	87	34	49.5	20.8	18.1	0.36	6.0	5.2	0.10	Increased	
7	3.0–3.2	110	69	25.5	13.3	15.3	0.29	7.1	7.3	0.14	Normal	
8	3.6–3.9	127	—	12.4	14.2	18.0	0.29	10.8	13.7	0.22	Increased	
9	3.9–4.1	101	—	1.9 (1)	—	—	—	—	—	—	Normal	Am
10	3.8–4.3	138	204	7.7 (9)	9.3	12.8	0.21	7.1	9.8	0.16	Normal	
11	3.3–4.1	114	244	<1	12.0	13.7	0.22	12.0	13.7	0.22	Normal	Am
12	4.0–3.6	118	180	2.7 (3)	14.1	16.6	0.31	14.1	16.6	0.31	Normal	Am
13	3.7–4.1	85		9.5	10.0	8.5	0.16	7.0	5.9	0.12	Normal	
14	3.5–3.6	80	123	<1	8.5	6.8	0.15	8.5	5.8	0.15	Normal	Am
15	3.7–3.2	69	—	<1	—	—	—	—	—	—	Decreased	Am
16	3.9–4.4	105	116	<1	11.4	12.0	0.27	11.4	12.0	0.2	Normal	
Controls	≥ 4.0	127.9 ± 21.3	—	<1	9.0 ± 0.99	11.5 ± 2.45	0.17 ± 0.037	—	—	—	—	

ples (21). The following significance levels were used

x	Probably significant	0.05 > p > 0.01
xx	Significant	0.01 > p > 0.001
xxx	Highly significant	0.001 > p

Results

Since the acute and non-acute types of ulcerative colitis are quite different from a clinical viewpoint, each type will be considered separately as will the 3 cases in remission.

The group of cases with regional enteritis and jejunoileitis is fairly heterogeneous, and therefore statistical analyses have not been considered justified.

Serum-proteins

Hypoproteinaemia (<6.5 g/100 ml) was present in only 2 out of the 16 cases of ulcerative colitis. Both belonged to the group of acute cases.

Initial hypoalbuminaemia (<4 g/100 ml) occurred in all acute cases (3.0 ± 0.41

Table IV Pre- and postoperative data on patients nos. 4 11 and 17 in whom total colectomy and ileostomy were performed

Case no.	Period of invest.	TBV (TBV/m)	PV (PV/m)	RCV (RCV/m)	I.v alb.	E.v alb.	Degradation			Klv/Krt
							% of I.v pool/d	g/d	g/kg/d	
4	Preop.	3.78 (2.35)	2.80 (1 74)	0.98 (0.61)	81	—	10.7	8.7	0.17	3.28
	Postop.	4 79 (2.98)	3.21 (1.99)	1.58 (0.98)	159	91	5.8	8.1	0.13	1.27
11	Preop.	5.16 (2.93)	3.46 (1.97)	1 70 (0.97)	114	244	12.0	13.7	0.22	1.94
	Postop.	6.13 (3.50)	3.63 (2 06)	2.52 (1 43)	203	250	10.6	21.5	0.25	1.74
17	Preop.	—	—	—	—	—	—	—	—	—
	Postop.	5.31 (3 02)	3.45 (1.96)	1.86 (1 06)	180	231	8.1	14.6	0.21	1.84

g/100 ml) only in 1 did the initial values exceed 3.5 g/100 ml. The albumin concentration was fairly constant during the period of investigation except in cases 2 (first study) 3 and 4 (table III)

Five of the 7 non acute cases had hypoalbuminaemia (3 6±0 35 g/100 ml) in only 2 of whom was the concentration below 3 5 g/100 ml. The first and the last values of the albumin concentration during the period of investigation are recorded in table III.

The 3 patients in remission all had slight hypoalbuminaemia. In case 14 the concentration was constant while in case 15 a fall and in case 16 a rise was noted

The patients investigated postoperatively had normal and constant concentrations of albumin

Three of the 7 enteritis patients all fairly acutely ill, had relative hypoproteinaemia (cases 18, 20 and 21)

Only 2 of the 7 patients (cases 22 and 23) with enteritis had normal albumin concentration Table VI shows the first and the last value of albumin concentration in each case.

Blood, plasma and red-cell volumes

Patients with *acute ulcerative colitis* did not exhibit any significant changes of TBV either totally or per metre height or per kg body weight (table II)

Likewise the PV was not significantly altered totally or per metre height. Per kg body weight, on the other hand, a significant (x) increase was found on comparison with the controls (54.2±9 8 and 47.2±6.0 ml respectively) (table II)

RCV was unchanged totally and per kg body weight, but significantly (x) lowered per metre height (0 76±0.17 and 0.96±0 19 l respectively) (table II)

The only significant changes that occurred among the *non-acute cases* were rises in TBV and PV when related to body weight (x and xx respectively) (table II)

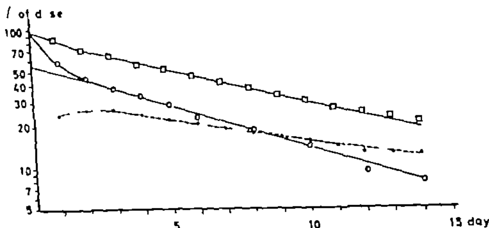


Fig. 1. Case 6. Acute relapse of ulcerative colitis with increased elimination of ^{131}I-albumin from plasma. ○——○ activity □——□ retained dose (total body activity) ● – – ● e. activity; T/2 activity 5 days (T/2 controls 15—20 days). I. /E. ratio 1:0.6 indicating markedly diminished extravascular pool (see table III)

The 3 patients in remission had relatively normal TBV and PV except patient no. 15 who had fairly low values. The RCV was slightly lower than normally.

Postoperatively there were normal values. When compared with preoperative volumes increases were found in TBV and then especially in the RCV while the increases in PV were moderate, 170 and 410 ml respectively (table IV).

Totally and relative to height, mainly normal values were obtained among the enteritis cases, but when related to body-weight slight rise was noted. Case 19 showed values higher than normal when examined postoperatively (table VI).

Intravascular albumin

The two main groups of ulcerative colitis together (cases 1—13) showed when compared with the controls, a significant decrease (xx) of the total amount of circulating albumin (103.5±21.7 and 127.9±21.3 g respectively) as well as of albumin relative to height (xxx) (60.3±11.5 and 75.2±10.3 g respectively).

In the *acute* group the same significances were found for the total intravascular albumin pool (xx) (93.7±22.1 g) as for that relative to height (xxx) (53.7±10.8 g) when compared with the controls. This contrasts with the comparison on a body-weight basis (x).

The *non-acute* cases showed no significant changes totally or by reference to different parameters.

Cases 14 and 15 (in remission) had low amounts of intravascular albumin per metre, while case 16 showed a quite normal value.

Postoperatively normal or slightly increased amounts were found (table IV).

In patients with *enteritis* the values were pathologically low in the acute phases, especially when referred to height, but not when referred to body-weight (table

Table IV Pre and postoperative data on patients nos. 4 11 and 17 in whom total colectomy and ileostomy were performed

Case no.	Period of invest.	TBV (TBV/m)	PV (PV/m)	RCV (RCV/m)	I.v alb.	E.v alb.	Degradation			Kiv/Krt
							% of i.v pool/d	g/d	g/kg/d	
4	Preop.	3.78 (2.35)	2.80 (1 74)	0.98 (0.61)	81	—	10.7	8.7	0.17	3.28
	Postop.	4.79 (2.98)	3.21 (1.99)	1.58 (0.98)	139	91	5.8	8.1	0.13	1.27
11	Preop.	5.16 (2.93)	3.46 (1.97)	1 70 (0.97)	114	244	12.0	13.7	0.22	1.34
	Postop.	6.15 (3.50)	3.63 (2.06)	2.52 (1 43)	203	250	10.6	21.5	0.23	1 74
17	Preop.	—	—	—	—	—	—	—	—	—
	Postop.	5.31 (3.02)	3.45 (1.96)	1.86 (1 06)	180	231	8.1	14.6	0.21	1.84

g/100 ml) only in 1 did the initial values exceed 3 5 g/100 ml The albumin concentration was fairly constant during the period of investigation, except in cases 2 (first study) 3 and 4 (table III)

Five of the 7 non-acute cases had hypoalbuminaemia (3 6±0 35 g/100 ml) in only 2 of whom was the concentration below 3 5 g/100 ml The first and the last values of the albumin concentration during the period of investigation are recorded in table III

The 3 patients in remission all had slight hypoalbuminaemia. In case 14 the concentration was constant, while in case 15 a fall and in case 16 a rise was noted.

The patients investigated postoperatively had normal and constant concentrations of albumin.

Three of the 7 enteritis patients all fairly acutely ill, had relative hypoproteinaemia (cases 18 20 and 21)

Only 2 of the 7 patients (cases 22 and 23) with enteritis had normal albumin concentration Table VI shows the first and the last value of albumin concentration in each case.

Blood plasma and red-cell volumes

Patients with *acute ulcerative colitis* did not exhibit any significant changes of TBV either totally or per metre height or per kg body weight (table II)

Likewise the PV was not significantly altered totally or per metre height. Per kg body-weight, on the other hand, a significant (x) increase was found on comparison with the controls (54.2±9 8 and 47 2±6.0 ml respectively) (table II)

RCV was unchanged totally and per kg body weight, but significantly (x) lowered per metre height (0 76±0 17 and 0 96±0 19 l respectively) (table II)

The only significant changes that occurred among the *non-acute cases* were rises in TBV and PV when related to body weight (x and xx respectively) (table II)

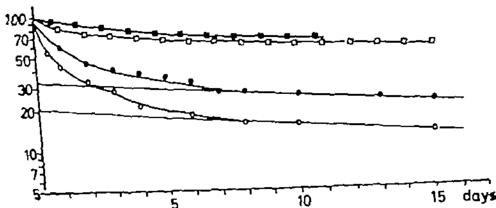


Fig. 3. Case 11. Elimination of ¹³¹I-labelled albumin in patient with non-acute ulcerative colitis pre and postoperatively (total colectomy + ileostomy). Symbols as in fig. 2. T/2 I. preop. 20 d. T/2 I.v postop. 22.5 d.

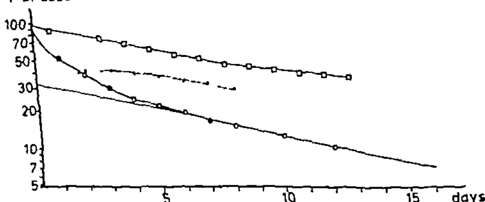


Fig. 4. Case 18. Subacute Jejunoileitis with increased elimination of ¹³¹I-albumin from plasma. T/2 I 2 d. I/E ratio 1 : 1.2 indicating the albumin deficit to be extravascular as well as intravascular. At the end some extravascular retention of the label seems to occur.

Faecal excretion of ¹³¹I-albumin (radioactivity)

Losses of radioactivity as a percentage of administered doses are given in table III. It will be seen that they varied between 5.8 and 49.5 % in the *acute cases of ulcerative colitis* during 6—11 days of collection. The normal loss is <1 % in the corresponding period of time. The highest value was recorded in case 6, in which the guaic test was strongly positive; however in cases 2 and 3 with 32.5 and 25.1 % respectively of the dose in the stools, no or negligible bleeding occurred.

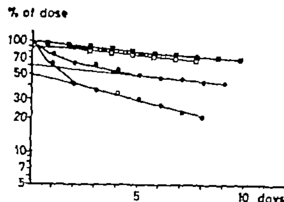


Fig 2. Case 4 Elimination of ¹³¹I-albumin in a patient with acute relapse of ulcerative colitis (treated with cortisone) pre and postoperatively (total colectomy + ileostomy) White symbols represent the preoperative and black symbols the postoperative investigation. The curves indicate a delayed *steady state* with extravascular retention of albumin preoperatively Postoperatively (see table IV) there is a steady state, but an e v pool that is smaller than the i.v pool. T/2 i v preop. 6.5 d T/2 i.v *postop.* 20 d.

○——○ ●——● i.v activity □——□ ■——■ retained dose.

VI) Patients who were not acutely ill had normal values. In patient no 19 who in the acute stage had a low intra vascular pool a normal value was re corded postoperatively

Degradation and losses of albumin

Only 1 patient with acute *ulcerative colitis* (case 1) was investigated with Amersham's ¹³¹I-albumin This patient had a degradation of 26 % which signi fied an increase as the value for the controls was 13.9±3.29 %

In the other patients (case 2—6) the albumin degradation was increased to 12 7—20 8 % except in case 4 where a value of 10 7 % was found (table III and figs. 1 and 2) In controls the degradation was 9 0±0 99 % Expressed in g and g/kg the degradation was normal to raised (table III)

If the faecal losses of activity are ex cluded when estimating the degradation (true degradation) normal or subnormal values are obtained (table III)

The non acute cases showed increased values immediately after acute relapses, otherwise normal values were found (table III and fig 3)

In two of the cases in remission the degradation could be determined. Case 14 showed a normal value, while case 16 had a slight rise.

Cases 4 and 11 (also studied pre operatively) as well as case 17 showed normal or lowered degradation post operatively (table IV figs. 2 and 3)

Among the *enteritis* cases (table VI and fig 4) case 18 with subacute jejunoileitis had the highest catabolic rate — 23 8 % or 18.3 g daily In this determination the albuminuria of 1 ‰ is not taken into account. In case 19 the study in the wet ileostomy period did not give any reliable numerical data, partly because of the im possibility of making a quantitative col lection of discharge from the "wet ileostomy The slope of the curve, how ever indicated an increased catabolism (possibly a pathological distribution as well) in marked contrast with the results of the study after total colectomy

In case 20 the investigation could only demonstrate an increased initial extra vascular distribution and indicate a raised catabolism as the plasma activity on the 5th day was only 28 8 % of the initial value.

In case 21 the degradation probably was *slightly increased in the first investigation.* In the second one, there was normal de gradation, without any faecal leakage.

Cases 22 23 and 24 had virtually normal degradation (9 4—11 1 %)

qualitative figures were achieved in case 18. A normal percentage amount of protein-bound activity (29—34 %) was found in the intestinal juice, but the non-precipitable fraction was increased (60—64 % normally <36 %) In the gastric juice, the protein-bound and non-precipitable fractions of activity were normal 42—48 % and 0—9 % respectively

Electrophoresis and determination of the radioactivity in the paper-strips showed the presence of albumin with the highest peak of activity in the albumin area. This peak was prominent in the intestinal but not in the gastric sample (fig 5)

Other investigations

The tests for liver function were normal in all but 2 cases (cases 11 and 24) The liver biopsy made in case 9 did not show any pathological changes.

Malabsorption tests performed in cases 8, 12, 15 and 20 were all normal.

In case 10 the faecal nitrogen content was increased, varying between 2.4—4 g/day (normally ~ 1.5 g) This means a loss of 15—25.0 g of protein per day The isotope study indicated a faecal loss of 3 g of albumin in the stools per day on the assumption that all activity represented albumin. Thus only a minor part of the nitrogen loss by this route can be ascribed to albumin.

Distribution of albumin

In the controls Kiv/Krt for Amersham albumin was 1 76±0.32 and for Abbott albumin 1 49±0 19 The latter ratio is also valid for the ^{131}I-albumin of our own preparation. In 5 of the acute cases of ulcerative colitis Kiv/Krt could be determined. In all, excluding case 4 normal values were found (1.33—1 78) In case 4 it was 3.28 (table V and fig 2)

Table V Albumin distribution expressed as quotient between elimination constants for intravascular and total retained activity (Kiv/Krt) in the patients with ulcerative colitis (A) and enteritis (B) Am = ^{131}I-albumin Amersham

	Cases	Kiv/Krt	
A.			
Acute	1	1.37	Am
	2	1.33	
	3	1.34	
	4	3.28	
	5	1 78	
	6	1.31	
Non-acute		1 18	
	8	2.68	
	9	—	Am
	10	1 79	
	11	1.94	Am
	12	1 14	Am
	13	2.38	
In remission	14	2.47	Am
	15	—	Am
	16	0.92	
B.	18	1.06	
	19 (postop.)	1 49	Am
	20	—	
	21	3.94	
	22	1.83	
	23	1 42	
	24	0.98	

In the non-acute group Kiv/Krt was normal in cases 7 10, 11 and 12 and raised in cases 8 and 13. In case 9 no value could be obtained, as some faecal collections were incomplete (table V)

In remission (cases 14 and 16) no certain pathological trends were observed 2.47 (Am) and 0.92 (Abb)

Postoperatively Kiv/Krt values were normal, even in case 4 with a preoperatively high value (table IV figs. 2 and 3)

Among the jejunitis and ileitis cases there were no certain pathological trends in

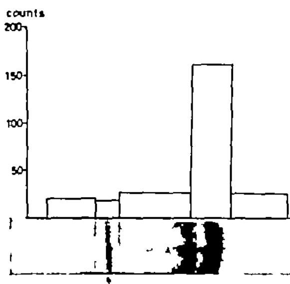


Fig 5 The distribution of radioactivity in electropherogram of jejunal juice from case 18 with jejunoileitis. The peak of radioactivity is in the albumin area.

The *non-acute cases* had smaller faecal losses ranging from 1 to 23 5 % In two patients (cases 9 and 12) however the collection time was too short, which probably means that the total catabolic rates in these cases are too low

Expressed quantitatively these values correspond to 3 0—13 0 g of albumin lost per day through the diseased bowel in the acute cases, on the presupposition that all radioactivity is equivalent to ¹I albumin In the non-acute cases the corresponding values are 0—8 g per day

In the remitting cases <1 / of activity was found in the stools (table III) No pathological leakage was revealed in the operated cases, i.e. a maximum of 1 / of the given dose was found in the discharges from the ileostomies

In two cases the amount of protein bound activity in faeces was determined 55 and 65 % were found to be protein bound

In case 7 the faecal excretion of ¹ I iodide was also investigated one month after the albumin study The ¹³¹I albumin study showed an excretion of 23.5 % of the administered dose into the faeces in 11 days, or a mean of 6.2 % of the intravascular activity per day while the corresponding mean for urine was 7 1 % When ¹³¹I iodide was given intravenously 48.6 % of the given dose was excreted in the urine and only 0 7 % in the stools on the first day and 8.6 % and 0 1 % respectively on the second day

No or very slight activity was found in the stools of the patients with *jejunitis and ileitis* (table VI) Screening test on the discharges from the wet ileostomy in case 19 demonstrated activity exceeding the average normal No activity was found in the stools from the permanent ileostomy

Only cases 22 and 23 had increased faecal activity of 7 4 and 1 3 % respectively corresponding to 2.5 and 0.6 g of albumin daily It should be pointed out here that these 2 patients had undergone ileocaecal resections and thus had shorter colonic passage than the others.

Case 24 in a clinically quiescent period but with a perityphlitic abscess showed no leakage and normal catabolism. The same was found postoperatively in case 19 (with an ileostomy)

Intestinal and gastric juices

Several attempts have been made in normals to determine the normal leakage of albumin into the intestine quantitatively by means of double balloon tubes, but because of several reasons without success. The main reason has been the small but inevitable distension of the intestine, caused by the balloons, which have given antiperistaltic movements of the balloons in the intestine, vomiting and subjective discomfort such as to necessitate interruption of the study Thus only

Abbreviations as in tables II and III

I. alb. (g/m; g/kg)	E.v. Ih.	Faecal loss of r.a. in % of dose	Degradation			Calculated synthesis of albumin
			% of I. pool/d	g/d	g/d/kg	
77 (43.8; 1.33)	92	< 1	23.8	18.3	0.32	Increased
96 (57.1; 2.39)	—	< 1	—	—	—	—
129 (76.6; 2.22)	149	1	7.7	9.9	0.17	Normal
55 (32.5; 1.28)	—	—	—	—	—	—
85 (50.6; 1.65)	—	< 1	—	—	—	—
81 (48.2; 1.76)	No steady state	< 1	9.9	7.9	0.16	Normal
118 (67.8; 1.64)	158	7.4	10.1	11.9	0.17	Normal-slightly decreased
145 (89.0; 2.30)	148	1.3	9.4	13.6	0.22	Normal
67 (42.0; 1.32)	109	< 1	11.1	7.4	0.17	Decreased
127.9 ± 21.3 (75.2 ± 10.5; 1.94 ± 0.31)	—	< 1	9.0 ± 0.99	11.5 ± 2.7	0.17 ± 0.037	—

no certain increase of synthesis. In case 4 the estimation is uncertain, but if the pathological distribution is taken into account the synthesis will at least be normal.

Case 7 shows a synthesis at least normal while in case 8 the high catabolism of 18 g/day was nearly compensated, which means an increased synthesis. In cases 9 and 12 the synthesis must be within normal limits when related to the serum-albumin level and the increased degradation.

Cases 10, 11 and 13 with increasing albumin levels and normal or slightly increased catabolism also indicate a slight rise in the production of albumin.

In case 11 the liver-function tests and the deduced estimation of albumin synthesis are controversial.

Two of the patients in remission showed a normal production of albumin, but in case 15 no exact catabolic rate was obtained and in spite of the remission and the absence of leakage a decrease of albumin concentration occurred, possibly

Table VI Diagnosis and clinical picture in the enteritis cases Data on the albumin situation are given.

Case no. Sex	Clinical picture	TBV (TBV/m)	PV (PV/m)	Alb. conc. g/100 ml initially and at end of invest.
18 ♂	Subacute jejunoileitis. Albuminuria 1°/₀₀	4.35 (2.47)	2.87 (1.63)	2.7–3.3
19 ♂	Terminal ileitis involving colon and rectum. Permanent and "wet" ileostomies	5.07 (3.02)	2.99 (1.78)	2.3
	6 months after ileal resection and total colectomy	6.11 (3.64)	3.79 (2.26)	3.2–3.8
20 ♂	Terminal ileitis death on 5th day of investigation from pulmonary embolism	3.53 (2.09)	2.61 (1.54)	2.1–2.1
21 ♀	Crohn's disease in duodenum and jejunum	4.94 (2.94)	3.56 (2.12)	2.4–2.7
	With multiple strictures. Half a year later cancer of the uterine neck with metastasis was found	4.64 (2.75)	3.11 (1.85)	2.6–3.3
22 ♂	Earlier ileocoecal resection. Now recurrence in terminal ileum. Diarrhoeas	5.32 (3.06)	3.46 (1.99)	4.0–3.4
23 ♂	Earlier ileocoecal resection. Now recurrence in terminal ileum. Diarrhoeas	4.44 (2.72)	3.02 (1.85)	4.8–5.0
24 ♀	Terminal ileitis also involving ascending colon. Quiescent. Perityphlitic abscess	3.38 (2.12)	2.46 (1.55)	2.7–2.8
	Controls	4 47 ± 0.80 (2.79 ± 0.37)	3.10 ± 0.45 (1.82 ± 0.21)	≥ 4 0

distribution except in case 21 during the second investigation, in which an extra vascular retention of activity was registered (table V B)

Production of albumin

Empirically it is possible to estimate the synthesis of albumin if the intra vascular albumin content is constant. This implies constant albumin concentration and constant plasma volume. Continuous determinations of plasma volume with conventional isotopic methods could not be made. It was however assumed to be constant, an assumption which is not inconsistent with the figures given for the plasma volumes in the different stages of the disease. The estimations are recorded in tables III and VI

Cases 2 and 6 with a catabolism of 19 5 and 18.1 g per day and no changes in albumin concentration illustrate an increased production of albumin compensating for the increased catabolism In cases 3 and 5 the catabolism was almost wholly compensated which means

Abbreviations as in tables II and III

I.v. alb. (g/m; g/kg)	E. alb.	Faecal loss of [illegible] a. in % of dose	Degradation			Calculated synthesis of albumin
			% of I.v pool/d	g/d	g/d/kg	
77 (43.8; 1.33)	92	< 1	23.8	18.3	0.32	Increased
96 (57.1; 2.29)	—	< 1	—	—	—	—
129 (76.6; 2.27)	149	1	7.7	9.9	0.17	Normal
55 (32.5; 1.28)	—	—	—	—	—	—
85 (50.6; 1.63)	—	< 1	—	—	—	—
81 (46.2; 1.76)	No steady state	< 1	9.9	7.3	0.16	Normal
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145 (85.0; 2.30)	149	1.3	9.4	13.6	0.22	Normal
67 (42.0; 1.32)	109	< 1	11.1	7.4	0.17	Decreased
127.9 ± 21.3 (73.2 ± 10.5; 1.94 ± 0.31)	—	< 1	9.8 ± 0.99	11.5 ± 2.75	0.17 ± 0.037	—

no certain increase of synthesis. In case 4 the estimation is uncertain, but if the pathological distribution is taken into account the synthesis will at least be normal.

Case 7 shows a synthesis at least normal, while in case 8 the high catabolism of 18 g/day was nearly compensated, which means an increased synthesis. In cases 9 and 12 the synthesis must be within normal limits when related to the serum-albumin level and the increased degradation.

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Two of the patients in remission showed a normal production of albumin, but in case 15 no exact catabolic rate was obtained, and in spite of the remission and the absence of leakage a decrease of albumin concentration occurred, possibly

indicating an insufficient production of albumin

Among the patients with enteritis case 18 (table VI) obviously had a synthesis surpassing the catabolism since in spite of a degradation of 18.3 g/day the concentration of albumin in plasma increased The other patients presented a roughly normal production of albumin except case 22 in which a falling albumin concentration indicated insufficient synthesis, as also in case 24 with acute-chronic infection and normal catabolism.

Discussion

Ulcerative colitis and regional enteritis are often accompanied by hypoproteinaemia (1 4 20 24) A certain parallelism between the degree of hypoproteinaemia and the extension character and duration of the disease has been proposed by Jankelson and McClure (12) and Ross and Swarts (20) an opinion not shared by others (1 23) Nevertheless a parallelism between albumin concentration and remissions and relapses of the disease has been pointed out by Spellberg (23) and Spellberg et al (24)

Classification of cases of ulcerative colitis is rather difficult. The frequency of diarrhoeas is not always an adequate or the only expression for the severity of the actual phase of the disease.

The patient s general condition and appearance, appetite, weight-gain or weight loss must also be taken into consideration

The division of our cases into different groups is based on these criteria. Therefore, some of the patients in the non acute group have fairly frequent diarrhoeas, but are in good general condition, have good appetite, and are gaining weight.

Hypoproteinaemia is thus not ubiquitous. It was found in only 2 of the acute colitis cases while 3 of the enteritis cases, all acute, showed values below 6.5 g/100 ml. The hypoalbuminaemia, on the other hand, was more accentuated in the acute group than in the non-acute. The same was seen among the patients with enteritis. Thus the degree of hypoalbuminaemia can be an index of the actual stage of the disease and similarly alterations in one direction or the other may indicate exacerbation or improvement.

Blood, plasma and red-cell volumes

Steinfeld et al. (26) reported increased plasma volumes in inactive ulcerative colitis and Crohn s disease. Their values are referred to body weight.

Steinfeld writes "Patients with hypoalbuminaemia may have subnormal serum albumin levels because of an expanded plasma volume, redistribution of albumin between the intra and extra vascular spaces, or a true subnormal total body albumin pool as a decreased albumin synthesis or excess albumin catabolism or loss

The problems concerning weight loss are the same as in cancer of the stomach, where fairly great weight losses have no significant influence on the blood or plasma volumes (31) This leads to a relative increase of the volumes, when correlated to body weight. But when a fixed parameter as height is used no changes are found. The results in this investigation demonstrate that no important changes of plasma volumes occur in any of the colitis groups or in patients with enteritis.

As the plasma volume is not significantly altered the hypoalbuminaemia cannot primarily be the result of a dilution leading to an increase of the plasma volume, but must have some other cause. The other reasons mentioned by Stein-

feld are valid here, i.e. increased catabolism and losses, lowered production, and possibly redistribution between intra and extravascular spaces. With a decreasing intravascular albumin pool the body mobilizes other mechanisms to maintain plasma volume.

It seems that the maintenance of blood and plasma volumes is no great problem in these diseases, the fulminant types not being taken into consideration. The problem concerns the colloids and certainly also the red-cell volume which is often subnormal.

The mechanism of this maintenance of the plasma volume is obscure and falls outside the scope of this investigation.

Intravascular albumin

The significant decrease of the intravascular albumin pool in the whole material as well as in the acute cases of colitis indicates increased catabolism with or without losses or lowered production of albumin, and sometimes a pathological distribution.

In the non-acute cases the hypoalbuminaemia is reflected by lower numerical values, but no significant difference is found when compared with the controls.

The normal intravascular pools in the operated cases indicate that the process inducing hypoalbuminaemia has been eliminated and also that there is a normal synthesis of albumin.

Cases in remission, on the other hand, do not necessarily show normal values for intravascular albumin, as is seen from cases 14 and 15. The possible reason for this will be discussed later.

Degradation and loss of albumin

This behaviour of the intravascular albumin pool is well mirrored in the degradation values found in these studies by means of ^{131}I-albumin. Increased degradation of albumin has been demonstrated earlier by Steinfeld et al. (26) Birke et al. (3) Wetterfors et al. (30). Faecal loss of radioactivity in ulcerative colitis and regional enteritis was first observed by Steinfeld et al. (25). As early as 1937 however Welch et al (29) postulated exudation of proteins through the pathologically changed bowel wall. Soergel and Ingelfinger (22) by immunoelectrophoresis demonstrated the presence of albumin and gammaglobulin in the rectal mucus from patients with ulcerative colitis.

If the faecal losses are excluded in estimating degradation rates, normal or subnormal values are found in the patients with colitis. This means that the "true" catabolism of albumin as a percentage is at least normal.

That degradation occurs on a percentage basis has been pointed out earlier (10, 13, 17, 31). But does all faecal radioactivity represent lost albumin or are degradation products also excreted by this pathological route. Steinfeld et al. found ~ 50 % of the activity in the stools to be protein-bound, and the same amount was found in our examined cases. The excretory capacity of the large bowel for ^{131}I-iodide in ulcerative colitis seemed to be very small as judged from the second study of case 7. This indicates that the percentage of albumin-degradation products containing ^{131}I-iodide in the stools is low. The non-protein-bound activity, at least the main part of it, is thus probably derived from local bacterial decomposition of the excreted protein.

That no activity is discharged from the ileum to the colon (when no malabsorption is present) is shown by the normal content of radioactivity in the ileostomy

stools in the operated patients and in the patient with a permanent as well as a wet ileostomy This fact gives further evidence of the colonic location of the leakage. Case 12 with a healthy ileum and diseased rectum showed increased faecal activity indicating rectal leakage as there was no evidence of malabsorption

The results presented here demonstrate an increased percentage degradation (i.e. the sum of "true catabolism and losses) of albumin especially in patients with acute relapses of ulcerative colitis while in the non acute cases slightly increased or normal values were obtained. This difference indicates diverging characters of the two phases of the disease.

There seems to exist no direct or inverse correlation between the duration of the disease and the size of the intravascular pool or the losses of albumin (or degradation) From the results in the group of acute relapses it may be postulated that patients with the acute fulminant onset are or will be still worse as regards the albumin pools.

As the colon was partially engaged in only a few of the cases investigated here, it is impossible to state whether the extension of the process and its location orally or distally also affects the magnitude of the albumin losses.

In the enteritis cases it was not possible to confirm Steinfeld s et al observation on the increased faecal activity even in the acute cases. But this probably depends on the character of our investigated cases Yet in 2 patients with fairly benign relapses of regional enteritis, where caecal resections with ileotransversostomies had been performed earlier increases in faecal activity were found As the bowel was healthy in these persons, the leakage would be iliac in origin but the absolute absorptive capacity of the large bowel was probably decreased because of its shortness. That the colon is able to absorb iodine has been well documented by Cohn (6)

In order to establish the increased leakage of albumin in a case of jejunoileitis with no activity in the faeces but increased degradation, jejunal intubation was performed As no segmental occlusion could be made, the information obtained was not quantitative, but indirectly it indicated an increased jejunal leakage.

Distribution of albumin

In using Campbell s method of determining the extravascular pool it is generally assumed that a steady state and possibly also equilibrium occur during first week of investigation with ^{131}I albumin. Usually this is the case but, as has been pointed out for cancer of the stomach (31) it applies only to those cases where the curves describing intravascular and total body activity are parallel or nearly parallel. The same holds for Matthews method for analysis of catabolism and distribution.

Kiv/Krt is within the normal range in most of the acute cases, thus indicating a steady state with complete mixing between the different pools within a reasonable period of time. Decreased extravascular pools of albumin together with the low intravascular ones indicate a generally decreased total exchangeable albumin pool of the body In case 4 however a high Kiv/Krt as well as a normal catabolism were registered. A deteriorating general condition necessitated operation and the investigation was thus interrupted on the 7th day But still the slopes of the curves on the 6th to 7th day indicated a delayed steady state, with a continuous net transport of ^{131}I-albumin

extravascularly. Possible explanations of this are the rather toxic state and/or the cortisone treatment. The same phenomenon was seen in two of the non-acute cases (6 and 13). In these cases the explanation is still more obscure, but could be a restitution of the extravascular pools (i.e. restitutional trapping of albumin extravascularly). In the other non-acute cases there were no significantly decreased extravascular pools.

Production of albumin

Before the studies of Sternfeld et al. it was proposed that insufficiency of the liver with impaired production of albumin was the main cause of hypoalbuminaemia (see Introduction).

To evaluate the albumin-production capacity of the liver by present indirect methods, it must be assumed that the plasma volume is constant throughout the investigation. The data presented here support this assumption. Thus, a constant albumin concentration in steady state signifies that synthesis balances catabolism. Because of the variability of body-weights it seems safer to deduce the synthesis from g/day than from g/day/kg.

This analysis showed that the synthesis of albumin in the liver was generally not disturbed and that it was increased in a few cases. In those patients in whom liver function was investigated by ordinary routine methods, no abnormalities were found except in two. In one of these, the findings were not consistent with the deduced synthesis of albumin, while in the other with a subacute infectious complication, liver-function tests suggested impairment and the albumin-synthetizing capacity was decreased. This case illustrated an observation made earlier by Gerzen (11) and Jarnum and Lassen (14) who found that in acute and chronic infections the catabolism of albumin is usually normal and a decreased synthesis seems to be the main reason for hypoalbuminaemia.

In the second case with a probably impaired synthesis the liver-function tests were normal. Thus the value of liver function tests seems to be doubtful in estimating the albumin-synthetizing capacity of the liver in patients with ulcerative colitis and Crohn's disease.

Summary

Ulcerative colitis and some kinds of enteritis are occasionally accompanied by a relative hypoproteinaemia, but nearly always by a relative hypoalbumineamia.

The degree of hypoalbuminaemia depends upon the actual stage of the disease and is pronounced in acute relapses. It does not seem to be correlated to the duration of the disease.

Blood and plasma volumes are in general well maintained throughout the different stages of the disease when referred to fixed parameters. The red-cell volume may be decreased. Post operatively a slight increase of the plasma volume may occur but the main increase concerns the red-cell volume.

The extravascular albumin pools are significantly decreased in acute relapsing cases.

The extravascular pools are also decreased in those cases, where no pathological distribution occurs. In non-acute cases the decrease of the pools is not so marked.

The degradation (true + loss) of albumin is increased in the acute stage and slightly increased or normal in the non-acute and remitting stages. This is attributed mainly to varying degree of *albumin losses through the diseased bowel wall.*

The albumin loss in the faeces is not proportional to the frequency of diarrhoeas.

stools in the operated patients and in the patient with a permanent as well as a wet ileostomy This fact gives further evidence of the colonic location of the leakage. Case 12 with a healthy ileum and diseased rectum showed increased faecal activity indicating rectal leakage as there was no evidence of malabsorption

The results presented here demonstrate an increased percentage degradation (i.e. the sum of "true catabolism and losses) of albumin especially in patients with acute relapses of ulcerative colitis while in the non-acute cases slightly increased or normal values were obtained. This difference indicates diverging characters of the two phases of the disease.

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Pathological distribution extravascularly may occur in some cases The explanation of this is not quite clear

The synthesis of albumin is normal or slightly increased though sometimes it may be decreased Thus, the synthesis is of no aetiological importance, but is incapable of compensating *for great losses of albumin.*

Malabsorption probably does not play any essential role in the causation of hypoalbuminaemia.

After total colectomy a full restitution to normal is achieved.

In acute as well as in non acute enteritis (unspecific and Crohn s disease) no evidence of faecal leakage was found probably indicating ileac or colonic absorption (of the label) The intestinal leakage *in these cases is thus only* indirectly evidenced.

As for malabsorption in enteritis it was not thoroughly investigated, but indirectly the normal or increased synthesis of albumin in some cases contradicts the importance of malabsorption in hypoalbuminaemia.

One patient operated upon was quite normal 6 months after resection of the diseased part of the intestinal tract.

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Ruptured Aneurysm of the Abdominal Aorta Involving the Kidneys

By

Leif G. Tallgren and Börje Kuhlbäck

The various symptoms and complications of aortic aneurysms have frequently been described in the medical literature. This report is mainly concerned with ruptured abdominal aortic aneurysms simulating renal disease or involving, without symptoms, one of the kidneys. The diagnostic difficulties confronting the physician handling an acute situation caused by a previously unknown abdominal aneurysm may at times be unsurmountable. However experience has proved that the correct diagnosis is far from difficult if such a possibility is kept in mind. The complications of an aortic aneurysm are highly acute and loss of time may be disastrous. Recent progress in vascular surgery has furthermore made early diagnosis a matter of therapeutic importance. What previously has been an intellectual curiosity is today an issue of practical significance (16). Genitourinary manifestations are frequently reported in the studies of the symptomatology of abdominal aortic aneurysms (1, 3, 4). However only few cases have been described where acute renal failure has assumed the dominating role. The mechanism underlying the renal failure in the cases described has been either uni- or bilateral ureteral obstruction or involvement of the renal vessels (6, 10, 12).

The four cases described below were all admitted to surgical departments of various hospitals as acute surgical cases. Two of them were referred to us as cases of acute renal failures. Hemodialysis was performed upon one of these. All cases ended fatally and the correct diagnosis was verified or established by autopsy.

Case reports

Case 1. Male, retired economist, aged 69 had for several years been treated by a cardiologist for congestive heart failure which was fairly well compensated with digitalis. The patient had been able to maintain his usual daily activities without any disturbing subjective symptoms. By palpation the cardiologist had diagnosed an abdominal aortic aneurysm. This had, however, not received any further attention and was unknown to the physician in charge for the day. Two days

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gradually to 8.6 g/100 ml, while the plasma creatinine rose from 6.0 to 7.5 mg %. The serum proteins varied from 5.8 to 6.2 g %. With the exception of a slightly subnormal serum potassium level the electrolytes were in balance. Because of signs of hyperhydration he was kept on fluid restriction and his body weight was by this regime reduced by almost 2 kg.

Because no anamnestic explanation of his anuria could be found, some kind of mechanical obstruction was sought for. Cystoscopy and ureteral catheterization were carried out. The catheter could not be introduced into the right ureter. The left ureter could readily be passed and higher up the catheterization yielded an abundance of urine, admixed with blood. It was concluded that his anuria was mechanical and an aortic aneurysm was suspected. On the 5th day the patient went into irreversible shock and expired. At this very moment a diffuse tumor-like expansion was palpated in the right half of the abdomen.

At autopsy a dissecting aortic aneurysm was found. The aortic wall was dissected starting from the right renal artery on its caudal side and continuing upwards as far as the arch of aorta. The right kidney was displaced by the hematoma and was located *just underneath* the diaphragm. As a consequence the renal artery was almost curved in the cranial direction. Some degree of compression was exerted by the hematoma upon the renal vessels. The ureter was firmly stretched through this compressing hematoma. At the upper pole of the right kidney thin-walled cyst (diameter 1.5 × 2.5 cm), containing clear fluid, was found. The left kidney contained many small-sized cysts. The cortex of the right kidney was covered by some macroscopic bleedings. The macroscopic structure of the right kidney was not distinct. There was no thrombosis of either renal artery. Neither ureter could be considered obstructed.

Case 3. Widow of factory owner aged 84 who about 14 years ago had an acute attack of cholelithiasis. This had ever since been treated with antispasmolytic drugs. She had also suffered from high blood pressure and congestive heart failure. Both had been of mild character and were held in balance with Rauwolfia and digitalis drugs. A year ago an abdominal aortic aneurysm had been diagnosed.

The acute incidence started with the patient collapsing in a store. She was immediately admitted to the hospital, where a systolic blood pressure of 80 mm Hg was measured. A pulse rate of 60/min. was recorded. At palpation the patient complained of pain in the right side of the abdomen especially the right hypochondrium. There were no abnormal neurological signs.

ECG showed a ST-elevation and a negative T-wave in V In V the ST was depressed. There was a leukocytosis of 14,600 cells. Hemoglobin was 11.1 g/100 ml. On the 3rd day the plasma creatinine was 3.75 mg % and serum potassium 5.8 mEq/l. Urine output was not measured on the 1st day. The 2nd day no urine was produced. On the 3rd and 4th day there were 92 ml and no urine respectively. There was also proteinuria and E. coli was abundantly found in her urine as well as epithelial cells.

The patient's blood pressure was normalized by Aramine injections. On the 3rd day there was relapse, whereafter the blood pressure stayed between 70—100 mm Hg for 12 hours. On the 4th day she expired in a new circulatory collapse.

Autopsy was performed and the *findings* were left ventricular dilatation of the heart, cholelithiasis and cystitis chronica. Furthermore, in the region of the right kidney a hematoma measuring one liter was found. This retroperitoneal expansion extended from the diaphragm to the inguinal fold. The aortic aneurysm was found on the caudal side of the right renal artery. It had the size of a full-grown fist. The rupture had taken place on its dorsal side. The right renal artery passed through the hematoma. Compression of the renal vessels could not be excluded. The left renal artery was in its proximal end filled with soft atheromatous masses. Both kidneys were pale.

The histological picture of the kidneys showed interstitial fibrosis with advanced tubular destruction, and non-specific sclerosis. The glomeruli were almost intact. No acute changes were found. The findings were in agreement with those of chronic pyelonephritis. This had also *previously been diagnosed* clinically.

The thyroid gland showed lymphocytic infiltration (struma lymphomatosa?).

before the incident the patient had been in good health, working late preparing a lecture for an academic anniversary During this very occasion he was struck by a low back pain. There was also some discomfort in the hypogastric region. The pain suddenly became intense and he was forced to leave after delivering his lecture. He collapsed in the lobby A surgeon who was present found the patient in shock but still conscious. At arrival in the hospital blood pressure was 120/70 mm Hg Because of the patient's stressed condition no anamnestic data were available. ECG showed signs of myocardial infarction. A leukocytosis of 11,200 cells was present. No tumor like enlargement could be palpated in the abdominal region. This picture resembling an acute cardiac insult was, however kept in doubt by the absence of any substernal pain. The patient complained only of pain in the lumbar region, the right iliac fossa and the right hip

The patient expired on the 4th day after admittance. He received 11 blood transfusions each of 450 ml, including altogether about 1 000 ml used for the priming of the artificial kidney Hemoglobin values were by this regime kept fairly constant 1st day 13 9 2nd day (four times) i) 11.9 ii) 12 8 iii) 12.5, iv) 13 4 and the 3rd day 9 7 (Hb g/100 ml) The leukocyte count rose to 15,400 cells on the 2nd day

During the first day of hospitalization no urine was produced. On the 2nd, 3rd and 4th day there was 40 ml, 15 ml and none respectively On the 2nd day the plasma creatinine had risen to 5 9 mg % Potassium was 5 1 mEq/l and sodium 150 mEq/l

Hemodialysis was performed on the 4th day because of progressive uremia. The ruptured aneurysm had then already been diagnosed and surgical procedure was planned after the correction of the uremia. The hemodialysis was successful despite heparinization. The patient did however expire before surgical procedures were performed.

Autopsy verified the diagnosis. A hematoma containing about 1 5 l blood was found in the right retroperitoneal space filling an area from the diaphragm to the inguinal fold. The right kidney was burrowed in this mass. The sac of the aneurysm was found on the caudal side of the renal artery The arteries were open, but the right renal artery as well as the right ureter was surrounded by the hematoma. The possibility of compression could not be excluded. Both kidneys were pale and swollen. Microscopic study revealed necrosis of the epithelium of the proximal tubules. Spots of bleeding were found and areas of total cellular necrosis. In the collecting tubules there was cellular debris. There were no changes of older origin. The left kidney was histologically mildly affected by tubular necrosis. In addition some fibrosis was present denoting some degree of involution. In neither kidney were glomerular or vascular changes visible. Furthermore, focal necrosis was found histologically in the liver indicating acute parenchymatous degeneration. A grave myocardial fibrosis of older origin was shown in the heart.

Case 2. Male, typographer aged 66, had previously only suffered from a few attacks of common cold Up to the time of his acute illness the patient had carried out his usual daily activities and regarded himself as being in good health

He was suddenly taken ill with headache nausea and diffuse abdominal pain. This was during the first day located in the hypogastrium. On the 2nd day he lost consciousness because of circulatory collapse (systolic pressure 60 mm Hg) He was admitted to a surgical ward, where he received hydrocortisone and metaradrine bitartrate (Aramine) 500 ml blood and 3 500 ml infusions consisting of 5 % glucose, saline and invert sugar The plasma creatinine rose to 3.3 mg% Hemoglobin decreased to 9 1 g/100 ml. There was a leukocytosis of 15,400 cells. The patient had become anuric and a small catheter specimen showed proteinuria (Esbach value 10 per mille) On the second day of hospitalization the patient was referred to the renal ward because of his progressive uremia.

Upon arrival in the renal ward his ECG showed a slight, insignificant ST-depression. Chest X ray showed some degree of hyper hydration The heart was hypertrophic and the aorta showed marked sclerosis. At times the patient complained of pains in the right hypogastrium. These were easily aggravated by palpation. The patient died on the 4th day of hospitalization. The urine outputs on the 1st, 2nd, 3rd and 4th day were none, 180 ml, 350 ml and 700 ml respectively In the renal ward his hemoglobin was 10.4 falling

gradually to 8.6 g/100 ml, while the plasma creatinine rose from 6.0 to 7.5 mg %. The serum proteins varied from 5.8 to 6.2 g %. With the exception of a slightly subnormal serum potassium level the electrolytes were in balance. Because of signs of hyperhydration he was kept on fluid restriction and his body weight was by this regime reduced by almost 2 kg.

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Case 4 Male, retired scientist, aged 73, had two years earlier suffered from a heart failure. The acute situation started with obstipation and the following day he was struck by an intense abdominal pain. At arrival in the hospital the patient was conscious but no blood pressure could be measured. The pulse was superficial and only faint sounds could be auscultated from the heart. He received analgetics and there was some subjective improvement. The abdomen was distended. At palpation of the abdomen there were signs of intense pain, but no muscular defense in the abdominal wall was found. In the left hypogastrium a tumor like expansion was palpated. His blood pressure could not be corrected and he expired within few hours.

At autopsy the lungs showed emphysematic changes. The coronary vessels were sclerotic, but there were no macroscopic signs of degeneration of the heart. The left kidney was displaced in the cranial direction by a large hematoma. There was no involvement of the renal vessels. The ureter was free. The aorta showed high degree of atherosclerosis. On the caudal aspect of the left renal artery there was a large abdominal aneurysm (size 21 × 12 cm) The rupture measured 3 cm in diameter On the left side of the vertebral column there was a retroperitoneal hematoma measuring about 1 l.

The short and dramatic course of this case did not permit acute renal failure to develop At autopsy the kidneys showed no microscopic involvement except for mild senile changes.

Discussion and comments

Four cases of ruptured aneurysms of the abdominal aorta are reported They all presented diagnostic problems while attempted therapy was unsuccessful. Immediate surgical procedure was not carried out. One died within few hours. The other three managed to survive for four days under symptomatic, conservative therapy This confirms the elsewhere expressed experience that ruptured abdominal aneurysms are not necessarily fatal immediately (13)

In three cases acute renal failure occurred. Case No 1 developed acute tubular necrosis and acute parenchymatous degeneration of the liver as a result of the stressed condition due to circulatory collapse. In this case the kidney directly affected by the surrounding hematoma showed greater changes possibly due to involvement of the renal artery The two other cases showed displacement of the collateral kidney In case No 2 the mechanical involvement of the ureter and renal artery seemed to dominate.

Two cases had been diagnosed previously but vascular surgery had not been contemplated The diagnosis was in three cases made clinically in the acute situation. In case No 2 the diagnosis was carried out by analogy with case No. 1

While discovery of an abdominal aneurysm had no bearing upon therapy in earlier days, the situation is clearly changed today The treatment of aortic aneurysms by excision of the involved segment and its replacement with homograft or synthetic prosthesis is today established as a successful procedure in vascular surgery Because of a greater occurrence of atherosclerotic aneurysms as a result of increasing life span and of the catastrophic nature and lethal issue of a ruptured aneurysm, the right diagnosis should be made early (5 7 11 16)

The diagnosis of an abdominal aneurysm can be very simple. However many cases are exceedingly difficult, but with roentgenographic studies such as plain posterior anterior and lateral films, pyelographic studies of the kidneys and ureter barium studies of the alimentary tract and as a last measure aortograms the correct diagnosis is seldom missed (13)

In well-documented earlier studies and many recent ones the symptomatology

and physical examination of abdominal aneurysms have been clearly described. This stock of knowledge should not be allowed to fade. In short, any patient with abdominal pain of undetermined or indeterminate nature should be examined as having a possible aneurysm (2, 8, 9 13 15)

Summary

Recent advances in vascular surgery have made early and correct diagnosis of an abdominal aneurysm a matter of importance. The wellknown symptomatology should be reviewed Urologic or nephrologic symptoms sometimes occur as the dominating feature of the disease. Four cases of ruptured aneurysms with involvement of the collateral kidney are presented. The diminishing value of conservative management of a ruptured aneurysm and the necessity for rapid surgery are stressed

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Haemodynamic Data During Rest and Exercise for Patients who Have or Have not Been Able to Retain their Occupation after Myocardial Infarction

By

RAOUL MALMCRONA, GUN CRAMÉR and ED VARNAUSKAS

The return to work of patients with previous myocardial infarction has been analysed by Weiss et al. (16) Master et al. (9) Irralo et al. (3) Biörck (1) and Malmcrona et al. (8) The relation between work load and heart rate has been tested on the bicycle ergometer by Söderholm et al. (15) in patients after infarction. Cardiac output at rest and during exercise has been determined by Chapman et al. (2) in patients who were symptomless 6 months or longer after myocardial infarction. Müller and Rørvik (10) have studied the pulmonary circulation in patients with dyspnoea or anginal pain after myocardial infarction. There is no information available on whether patients who did not return to their previous work were haemodynamically worse than those who did return.

The present study was undertaken to illustrate the relation between working capacity i. e. ability or disability to return to work, and selected haemodynamic data at rest and during exercise on a bicycle ergometer.

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Material

The material consisted of two groups of patients with previous myocardial infarction. The first group (group I) consisted of 16 males with myocardial infarction 1—7 years previously all of whom had been able to return to work after the infarction and were still working at the time of the investigation. The other group (group II) comprised 8 males with myocardial infarction 3—11 years previously. They had not returned to full work after the infarction because of symptoms referable to the heart disease or had sickness pension. These patients were compared with healthy males described previously (7).

All patients had displayed clinical signs of myocardial infarction during the acute stage. In most patients the ECGs showed ST segment elevations and in due course development of pathological Q waves. Individual clinical data are given in table I. No ECGs were available from the acute stage of the patients nos. 15 and 23.

The mean age of the 16 patients in group I was 51 years. Eleven of them were labourers before the infarction. 4 of these had changed to physically less strenuous work before being examined. Before the infarction 5 had physically less strenuous work. At the follow up investigation two patients had exertional dyspnoea and two showed roentgenological signs of pulmonary congestion. Five patients had roentgenological heart size

Physical work before infarction	Physical work re-examination	Heart failure	Heart size (ml and ml/sqm BSA)	Pulmonary congestion	Digitalis medification	Effort angina	ECG	Electrocard. response during and after work
Yes	Yes	No	670/440	No	No	Yes	Pathol. Q and T waves	Normal
Yes	Yes	No	750/410	No	No	No	Pathol. Q and T waves	Normal
No	No	No	1,140/500	No	No	No	Pathol. Q wave	Normal
Yes	Yes	No	790/440	No	No	No	Pathol. Q wave	Normal
No	No	No	760/410	No	No	No	Pathol. Q wave	Normal
Yes	No	No	630/350	No	No	No	Normal	Normal
Yes	Yes	No	1,050/500	No	No	No	Pathol. Q and T waves	ST and T wave changes
Yes	Yes	No	930/540	Yes	No	Yes	Pathol. Q and T waves	Ventr. ES
No	No	No	820/460	No	No	No	Pathol. Q and T waves	ST and T wave changes
No	No	Dyspnea	980/570	No	No	Yes	Pathol. Q and T waves	ST and T wave changes
Yes	No	Dyspnea	830/430	Yes	No	No	Pathol. Q wave	ST and T wave changes
No	No	No	590/330	No	No	No	Normal	ST and T wave changes
Yes	Yes	No	810/470	No	No	No	Pathol. Q and T waves	ST and T wave changes
Yes	No	No	740/420	No	No	No	Pathol. Q and T waves	ST and T wave changes
Yes	No	No	560/330	No	No	Yes	Pathol. Q and T waves	ST and T wave changes
Yes	Yes	No	820/340	No	Yes	No	Pathol. Q and T waves	ST and T wave changes

Table I Clinical data for survivors of myocardial infarction with unlimited and limited working capacity

Pat.	Age (yrs)	Height (cm)	Weight (kg)	No. of myo-cardial infarctions	Time from 1st myo-cardial infarction to re-investigation (yrs)	Site of myocardial infarction	Occupation before infarction	Occupation at re-examination
Unlimited working capacity								
1	49	181	80.0	1	6 0/12	Posterior	Mechanic	Same
2	46	171	69.9	1	5 9/12	Anterior Posterior	Sail-maker	Same
3	51	190	99.5	1	4 7/12	Posterior	Salesman	Same
4	44	176	61.3	1	3 6/12	Anterior	Taxidriver	Same
5	53	175	71 7	1	3 5/12	Anterior	Builder contractor	Same
6	43	169	71 1	1	1 3/12	Anterior	Store-keeper	Same
7	45	174	93.2	1	4 5/12	Anterior	Fireman	Same
8	56	168	63.8	1	3 5/12	Posterior	Stoker	Freight lift man
9	55	194	77 4	1	6 8/12	Anterior	Accountant	Same
10	56	170	69 4	1	3 8/12	Posterior	Clerk	Same
11	54	173	75.2	1	3 8/12	Anterior	Dining-car waiter	Head waiter
12	52	181	59.8	1	5 0/12	Anterior	Engineer	Same
13	51	169	63	1	5 0/12	Anterior	Fire brigade officer	Same
14	49	167	69.6	2	2 5/12	Posterior	Store keeper	Same
15	53	168	63.0	1	5 1/12	Posterior	Mate	Store-keeper
16	59	173	67.3	1	1 1/12	Posterior	Builder	Same

Physical work before infarction	Physical work at re-examination	Heart failure	Heart size (ml and ml/sqm B.S.)	Pulmonary congestion	Digitalis medication	Effort angina	ECG	Electrocard. response during and after work
Yes	Yes	No	870/440	No	No	Yes	Pathol. Q and T waves	Normal
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Yes	Yes	No	1,030/500	No	No	No	Pathol. Q and T waves	ST and T wave changes
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15	53	168	63.0	1	5 1/12	Posterior	Mate	Store-keeper
16	59	173	67.3	1	1 1/12	Posterior	Builder	Same

Physical work before infarction	Physical work at reexamination	Heart failure	Heart size (ml and ml/sqm BSA)	Pulmonary congestion	Digitalis medication	Effort angina	ECG	Electrocard. response during and after work
Yes	Yes	No	670/440	No	No	Yes	Pathol. Q and T waves	Normal
Yes	Yes	No	750/410	No	No	No	Pathol. Q and T waves	Normal
No	No	No	1,140/500	No	No	N	Pathol. Q wave	Normal
Yes	Yes	No	750/440	No	No	No	Pathol. Q wave	Normal
No	No	No	760/410	No	No	No	Pathol. Q wave	Normal
Yes	No	No	630/350	No	No	No	Normal	Normal
Yes	Yes	No	1,050/500	N	No	No	Pathol. Q and T waves	ST and T wave changes
Yes	Yes	No	850/540	Yes	No	Yes	Pathol. Q and T waves	Ventr. ES
No	No	No	820/400	No	No	No	Pathol. Q and T waves	ST and T wave changes
No	No	Dyspnea	800/570	No	No	Yes	Pathol. Q and T waves	ST and T wave changes
Yes	No	Dyspnea	830/430	Yes	No	No	Pathol. Q wave	ST and T wave changes
No	No	No	590/330	No	No	No	Normal	ST and T wave changes
Yes	Yes	No	810/470	No	No	No	Pathol. Q and T waves	ST and T wave changes
Yes	No	No	740/420	No	No	No	Pathol. Q and T waves	ST and T wave changes
Yes	No	No	560/330	No	No	Yes	Pathol. Q and T waves	ST and T wave changes
Yes	Yes	No	620/340	No	Yes	No	Pathol. Q and T waves	ST and T wave changes

Table I (cont.)

Pat.	Age (yrs)	Height (cm)	Weight (kg)	No. of myocardial infarctions	Time from 1st myocardial infarction to re-investigation (yrs)	Site of myocardial infarction	Occupation before infarction	Occupation at re-examination
Limited working capacity								
17	55	168	72.1	1	4 7/12	Posterior	Mechanic	Sweeper
18	58	171	72.8	1	5 8/12	Anterior	Plumber	Filer
19	46	174	85.6	1	5 1/12	Posterior	Window cleaner	Pensioner
20	63	167	62.9	1	10 5/12	Anterior	Mechanic	Pensioner
21	64	171	63.5	2	11 0/12	Anterior	Builder	Pensioner
22	57	162	58.9	3	5 5/12	Anterior Posterior	Mechanic	Pensioner
23	58	172	59.4	1	4 6/12	Posterior	Paper mill worker	Pensioner
24	63	160	58.7	3	11 3/12	Anterior	Labourer	Pensioner

larger than 450 ml/sq m body surface area, as determined according to Jonsell (4). Four patients had effort angina. The ECG was normal at rest in 2 patients. The others had retained the pathological Q or Q and T waves. Exercise tolerance tests were performed and the ECGs during and after work analysed as described by Söderholm et al. (15). There was a pathological change of the ECG in 10 of the 16 patients.

The mean age of the 8 patients in group II was 58 years. All of them were labourers before the infarction. Six patients had been regarded as completely disabled due to the myocardial infarction and had received a continuous sickness pension. Two patients were able to work part-time in physically less strenuous jobs, and one patient (a window cleaner) with pension had his old work for shorter periods. At the follow-up 6 had dyspnoea on exertion. Three patients showed roentgenological signs of pulmonary congestion. Five patients had a roentgenological heart size larger than 450 ml/sq.m body surface area. Six patients had effort angina. All but one had persisting pathological Q or Q and T waves. No exercise tests were performed on two patients because of the electrocardiographic changes at rest. During or after exercise pathological changes of the ECG appeared in 3 of the 6 patients tested.

The group of normal individuals consisted of 14 men with a mean age of 51 years, previously described elsewhere (7). They were healthy as judged from case histories, physical examination and ECG. Five were labourers. Mean anthropometric data for the three groups are given in table II.

Physical work before infarction	Physical work at reexamination	Heart failure	Heart size (ml and ml/sqm BSA)	Pulmonary congestion	Digitalis medication	Effort angina	ECG	Electrocard. response during and after work
Yes	No	No	850/480	No	No	No	Normal	Normal
Yes	No	Dyspné	1 040/560	Yes	Yes	No	Pathol. Q and T waves	Not performed
Yes	No	Dyspné	900/450	No	No	Yes	Pathol. Q wave	Normal
Yes	No	Dyspné	650/380	N	No	Yes	Pathol. Q wave	Normal
Yes	No	Dyspné	960/550	Yes	Yes	Yes	Pathol. Q and T waves	ST and T wave changes
Yes	No	Dyspné	850/500	Yes	No	Yes	Pathol. Q and T waves	Not performed
Yes	No	No	650/360	No	No	Yes	Pathol. T wave	ST and T wave changes
Yes	No	Dyspné	900/570	No	Yes	Yes	Pathol. Q and T waves	ST and T wave changes

Methods

The patients were admitted to the hospital for the follow-up investigation. On the day of admission there was physical examination, routine blood tests were done and the patient performed an exercise test for evaluation of the electrocardiographic response to work. This was performed in the Department of Clinical Physiology (Head Prof A. Carlsten) by Assistant Professor B. Söderholm, M. D. A circulation study was made next morning with the patients fasting overnight. On the third day chest X-ray was taken. This was performed in the First Department of Roentgenology (Head Prof. S. R. Kjellberg). The patient was then discharged.

For the circulatory study catheters were inserted percutaneously into the brachial artery and cubital vein, according to Seldinger (13). The venous catheter was advanced to the subclavian vein. Intra-arterial blood pressures were registered by manometers of the variable inductance type. Cardiac output was determined by the dye-dilution technique with bromsulphalein as indicator (17). Expired air was collected in Douglas bag and analysed for oxygen (12). Arteriovenous oxygen difference was calculated from the cardiac output and oxygen consumption. Peripheral vascular resistance was calculated from the mean pressure in the brachial artery and the cardiac output.

During measurements at rest the patient was sitting in an arm chair. Work was performed on a bicycle ergometer. Collection

Table II Mean values of anthropometric data in normal persons and survivors of myocardial infarction with unlimited or limited working capacity

		Normal men	Patients with a myocardial infarction	
			Unlimited working capacity	Limited working capacity
Age	No.	14	16	8
	Mean	50.6	51.2	58.3
	Range	44—58	43—59	46—64
Height	No.	14	16	8
	Mean	174.6	174.8	169.4
	Range	166—187	167—194	160—177
Weight	No.	14	16	8
	Mean	75.1	72.1	69.5
	Range	62—99	58.1—99.5	58.7—85.6
Body surface area	No.	14	16	8
	Mean	1.91	1.88	1.80
	Range	1.72—2.16	1.72—2.30	1.61—2.03

of expired air began in the 6th to 7th minute of work and continued for 4—6 minutes. The cardiac output was determined in the 9th to 10th minute.

The first cardiac output determination was made 20 minutes after insertion of the catheters. If two work periods were used the patient rested 20 minutes between them.

The procedure and methods have been fully described in a previous paper (7).

Results

Circulatory data at rest

Data for individual patients are given in table III. All had sinus rhythm.

Means at rest for the two groups of patients and for the normal men are given in table IV.

The oxygen consumption and heart rates at rest were similar in all groups.

The cardiac output was similar in the normal persons and in the patients of group I but lower ($p < 0.05$) in the patients of group II.

The mean stroke volume was highest for the normal men, lower for the patients of group I and lowest for the patients of group II. The difference was significant ($p < 0.01$) only when the normal group was compared with the group II patients.

The arteriovenous oxygen difference was similar in the first two groups and lower ($p < 0.01$) in the patients of group II.

The arterial pressures were similar in all groups. The resistances were similar in the first two groups and higher in the patients of group II.

In the two groups of patients there were 9 patients with dyspnoea on exertion or signs of pulmonary stasis, 6 of whom were unable to work. The mean value of the cardiac output for these 9 patients was 4.89 l/min.

In the two groups there were 9 patients with a heart size of more than 450 ml/sq.m BSA. Their mean cardiac output was 4.98 l/min.

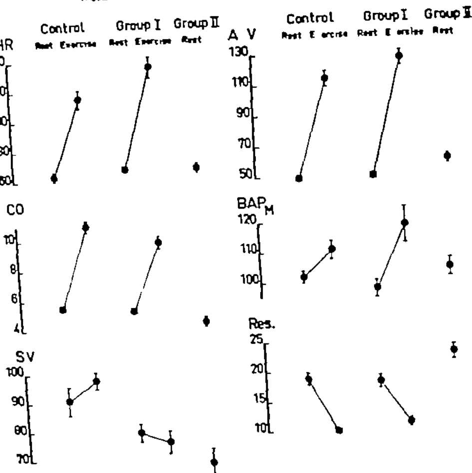


Fig 1 Mean values, and standard error of the mean, of haemodynamic data at rest and during exercise for control subjects and for patients who retained their work after myocardial infarction (group I) and at rest for patients with limited working capacity (group II)

HR heart rate, beats/min. CO cardiac output, l/min. SV stroke volume, ml. A-V arteriovenous oxygen difference, ml/l. BAP_M brachial arterial mean pressure mm Hg Res resistance, units. Exercise load 400 kpm/min.

In the two groups there were also 10 patients with angina of effort, 6 of whom were unable to work. The mean cardiac output for these 10 patients was 5 04 l/min

In the two groups there were 13 patients with pathological changes in the electrocardiogram during or after work. Their mean cardiac output was 4.99 l/min.

In group I there were 7 patients doing predominantly physical work. Their mean cardiac utput was 5.21 l/min. while the output of the 9 patients of group I doing white-collar work at reexamination was 5.37 l/min.

The best distinction between patients with high and low cardiac outputs was their ability to continue their employment.

Table III Circulatory data of survivors of myocardial infarction with unlimited or limited working capacity

Pat.	Rest sitting in an arm chair									Work sitting on a bicycle ergometer			
						Brachial arterial pressure							
	Heart rate (beats/min)	Oxygen consumption (ml/min)	Cardiac output (l/min)	Arteriovenous oxygen difference (ml/l)	Stroke volume (ml)	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	Resistance (units)	Work load (kpm/min)	Heart rat (beats/min)	Oxygen consumption (ml/min)	Cardiac output (l/min)
Unlimited working capacity													
1	69	298	6.2	48	89	118	61	85	13.8	—	—	—	—
2	55	251	4.6	55	84	—	—	90	15.7	200	103	949	7.9
	—	—	—	—	—	—	—	—	—	400	139	1,282	9.2
3	74	332	7.2	46	97	125	76	97	13.5	300	110	—	14.6
4	59	228	4.6	50	77	123	73	92	20.2	150	82	661	6.5
	—	—	—	—	—	—	—	—	—	300	105	936	8.7
5	68	270	5.8	47	85	123	69	90	15.6	200	98	864	8.0
	—	—	—	—	—	—	—	—	—	400	132	1,282	10.8
6	73	196	4.7	42	64	131	94	111	23.7	200	120	734	7.2
	76	203	4.8	43	63	132	93	110	23.2	400	167	1 110	8.1
7	81	346	5.5	63	68	153	96	122	22.3	200	123	1,069	8.5
8	65	—	7 1	—	110	139	78	98	13.8	—	—	—	—
	69	259	6.6	40	95	138	76	95	14.5	—	—	—	—
9	67	250	5.7	44	85	118	68	85	14.9	200	111	948	9.2
	—	—	—	—	—	—	—	—	—	400	135	1,288	10.0
10	63	292	5.5	53	87	135	72	98	17.9	150	82	819	8.3
11	68	220	4.4	51	64	156	91	115	26.4	200	130	1 138	9.2
	—	—	—	—	—	—	—	—	—	400	149	1,368	9.9
12	59	210	5.4	39	92	125	73	95	17.6	200	74	735	8 8
	56	207	5.3	39	95	123	74	93	17 4	400	101	1 147	12.0
13	64	232	4.4	53	68	127	77	97	22.3	200	98	793	7 0
14	—	—	—	—	—	—	—	—	—	200	113	882	8.3
	69	261	4.8	54	70	140	77	103	21 4	400	140	1,372	10.1
15	71	229	4.7	49	66	133	70	94	0.0	150	117	843	7 7
16	57	255	4.3	60	75	142	67	92	21 6	200	90	780	7.2
	—	—	—	—	—	—	—	—	—	400	116	1 106	9.3

ork sitting on bicycle ergometer						Estimated increase of heart rate, cardiac output and arterial pressures/100 ml oxygen consumption increase					Oxygen consumption increase used for calculation (ml/min)
		Brachial arterial pressure						Brachial arterial pressure			
Arteriovenous oxygen difference (ml/l)	Stroke volume (ml)	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	Resistance (units)	Heart rate (beats/min)	Cardiac output (l/min)	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	
—	—	—	—	—	—	—	—	—	—	—	—
119	77	—	—	103	13.0	6.9	0.43	—	—	—	1,031
139	66	—	—	102	11.1	—	—	—	—	—	—
—	133	156	82	110	7.5	—	—	—	—	—	—
102	79	140	82	105	16.2	6.5	0.38	2.7	1.0	1.6	700
108	82	142	80	103	11.9	—	—	—	—	—	—
108	82	150	76	106	13.2	6.3	0.49	3.4	2.0	2.9	1,012
119	82	178	89	119	11.0	—	—	—	—	—	—
102	90	140	100	121	16.8	10.1	0.38	2.2	2.0	1.1	910
137	49	152	112	121	14.9	—	—	—	—	—	—
126	68	—	—	—	—	5.8	0.42	—	—	—	723
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
104	82	147	78	105	11.3	6.6	0.41	2.6	1.2	2.0	1 036
129	74	145	80	106	10.6	—	—	—	—	—	—
94	101	153	80	112	13.5	3.6	0.53	3.4	1.5	2.7	527
124	71	207	115	156	16.9	7.2	0.48	—	—	4.2	1 148
139	64	—	—	160	16.2	—	—	—	—	—	—
84	118	140	74	104	11.9	4.6	0.70	4.3	0.9	3.0	938
96	125	164	82	122	10.2	—	—	—	—	—	—
113	71	144	80	107	15.3	6.1	0.47	3.0	0.5	1.8	561
107	73	155	82	110	13.3	6.4	0.48	3.4	0.5	1.0	1,111
156	72	178	83	114	11.3	—	—	—	—	—	—
110	66	172	88	126	16.4	7.5	0.48	6.3	2.9	5.2	616
108	80	165	68	100	13.9	6.9	0.59	4.9	1.1	2.0	851
119	80	184	76	109	11.7	—	—	—	—	—	—

Table III (cont.)

Pat.	Rest sitting in an arm chair									Work sitting on a bicycle ergometer			
	Heart rate (beats/min)	Oxygen consumption (ml/min)	Cardiac output (l/min)	Arteriovenous oxygen difference (ml/l)	Stroke volume (ml)	Brachial arterial pressure: Systolic (mm Hg)	Brachial arterial pressure: Diastolic (mm Hg)	Brachial arterial pressure: Mean (mm Hg)	Resistance (units)	Work load (kpm/min)	Heart rate (beats/min)	Oxygen consumption (ml/min)	Cardiac output (l/min)
Limited working capacity													
17	74	305	5.3	58	71	140	88	109	20.7	—	—	—	—
	—	—	—	—	—	—	—	—	—	400	120	1,259	10.5
18	50	275	4 0	70	79	115	59	78	19.7	—	—	—	—
	46	280	3.9	73	90	123	60	83	21.5	—	—	—	—
19	64	319	5.7	56	90	128	74	96	16.8	300	90	1 106	8.2
20	79	263	4 4	60	56	158	85	111	25.1	150	—	—	7.3
21	67	332	5.0	66	75	200	100	135	26.8	150	130	870	3.9
22	73	262	3.9	67	53	131	75	98	25.2	—	—	—	—
	67	257	3.5	73	53	124	70	90	25.5	—	—	—	—
23	62	203	3.8	53	61	118	73	92	24.2	150	80	—	6.0
24	69	220	4.6	48	67	177	96	127	27.6	200	105	880	8.8
	68	229	4.3	53	63	163	86	120	27.8	400	132	1,231	17 1

Circulatory data during work

Nine of the 16 patients in group I exercised at either 200 and 400 kpm/min or 150 and 300 kpm/min Five patients performed exercise at one work load and two did not exercise. Six of the 8 patients in group II exercised one of them at both 200 and 400 kpm/min Oxygen consumption was measured in 4 patients. The group is so small that the values will not be compared with those of the other two groups. Individual data during work are given in table III

Nine normal men and 8 patients in group I exercised at 400 kpm/min. The mean circulatory data for these two groups are given in table V

The oxygen consumption at a work load of 400 kpm/min. was similar in the normal men and the patients of group I The heart rate was higher ($p < 0.02$) for the patients The mean cardiac output tended to be lower for the patients. The stroke volume was lower ($p < 0.02$) The mean arteriovenous oxygen difference and the resistance tended to be higher in the patients. The arterial pressures were of the same order

Mean haemodynamic data at rest and during exercise for controls and group I patients and at rest for group II patients are visualized in fig 1

Work sitting on bicycle ergometer						Estimated increase of heart rate, cardiac output and arterial pressures/100 ml oxygen consumption increase					Oxygen consumption increase used for calculation (ml/min)
		Brachial arterial pressure						Brachial arterial pressure			
Arteriovenous oxygen difference (ml/l)	Stroke volume (ml)	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	Resistance (units)	Heart rate (beats/min)	Cardiac output (l/min)	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	
—	—	—	—	—	—	4.9	0.58	0.2	−0.2	−0.2	934
118	88	142	88	107	10.2	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
133	91	141	77	102	12.4	3.3	0.32	1.7	0.4	0.8	787
—	—	184	102	133	18.4	—	—	—	—	—	—
148	45	220	115	155	26.4	11.7	0.15	3.7	2.8	3.7	358
—	—	—	—		—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—	75	158	90	118	19.6	—	—	—	—	—	—
100	83	202	94	142	16.1	6.5	1.25	4.6	0.1	1.7	1,006
75	130	216	92	141	8.2	—	—	—	—	—	—

Calculation of increase in heart rate, cardiac output and brachial arterial pressure per 100 ml oxygen consumption increment made it possible to compare the whole group of 14 normal men with 13 patients of group I regarding heart rate and cardiac output, and with 11 regarding brachial arterial pressures. The mean oxygen consumption increment used for the calculation was 1,040 ml/min. in the normal men and 860 ml/min. in the patients.

The calculated mean increases in heart rate, cardiac output and brachial arterial pressures per 100 ml oxygen consumption increment and the incremental oxygen consumption used for calculation are given in table VI.

The increase in heart rate per 100 ml incremental oxygen consumption was 3.1 beats/min. in the normal men and 6.5 in the patients of group I ($p < 0.05$). The corresponding values for the cardiac output were 0.56 and 0.50 l/min. The increase in systolic pressure per 100 ml oxygen consumption increment was 2.6 mm Hg in the controls and 4.0 in the patients ($p < 0.02$). The increase in diastolic and mean pressures in the controls were 0.7 and 1.5 mm Hg while the corresponding values for the group I patients were 1.5 and 2.5 mm Hg ($p < 0.05$).

Table IV Mean values of circulatory data at rest in normal persons and survivors of myocardial infarction with unlimited or limited working capacity

		Normal men	Patients with a myocardial infarction	
			Unlimited working capacity	Limited working capacity
Oxygen consumption (ml/min)	No.	14	16	8
	Mean ± SE of mean	265 ± 6	258 ± 10	273 ± 16
	SD	22	42	47
Heart rate (beats/min)	No.	14	16	8
	Mean ± SE of mean	63 ± 3	66.6 ± 1.8	66.6 ± 3.3
	SD	11	7.1	9.3
Cardiac output (l/min)	No.	14	16	8
	Mean ± SE of mean	5.54 ± 0.23	5.30 ± 0.22	4.53 ± 0.26
	SD	0.87	0.89	0.74
				< 0.05
	Probabil. of diff.		< 0.01	
Stroke volume (ml)	No.	14	16	8
	Mean ± SE of mean	91 ± 5	80 ± 3	70 ± 5
	SD	18	13	13
	Probabil. of diff.		< 0.01	
Arteriovenous oxygen difference (ml/l)	No.	14	16	8
	Mean ± SE of mean	49.0 ± 1.8	49.7 ± 1.7	60.8 ± 2.8
	SD	6.8	6.7	7.8
				< 0.01
	Probabil. of diff.		< 0.01	
Brachial arterial pressure, systolic (mm Hg)	No.	14	15	8
	Mean ± SE of mean	139 ± 4	133 ± 3	145 ± 10
	SD	14	12	29
Brachial arterial pressure, diastolic (mm Hg)	No.	14	15	8
	Mean ± SE of mean	81 ± 2	76 ± 3	81 ± 5
	SD	7	10	13
Brachial arterial pressure, mean (mm Hg)	No.	14	16	8
	Mean ± SE of mean	102 ± 2	98 ± 3	105 ± 6
	SD	9	10	18
Resistance (units)	No.	14	16	8
	Mean ± SE of mean	19.0 ± 1.1	18.8 ± 1.0	23.4 ± 1.3
	SD	4.2	4.0	3.7
				< 0.02
	Probabil. of diff.		< 0.02	

Table V Comparison of haemodynamic data at work load of 400 kpm/min in normal old men and survivors of myocardial infarction with unlimited working capacity after a myocardial infarction

		Normal men	Myocardial infarction. Unlimited working capacity
Oxygen consumption (ml/min)	No.	9	8
	Mean ± SE of mean	1,232 ± 22	1,244 ± 33
	SD	67	100
Heart rate (beats/min)	No.	9	8
	Mean ± SE of mean	115 ± 6	135 ± 7
	SD	19	19
	Probabil. of diff.	< 0.02	
Cardiac output (l/min)	No.	9	8
	Mean ± SE of mean	11.0 ± 0.4	9.9 ± 0.4
	SD	1.3	1.2
Stroke volume (ml)	No.	9	8
	Mean ± SE of mean	98 ± 6	77 ± 8
	SD	19	22
	Probabil. of diff.	< 0.02	
Arteriovenous oxygen difference (ml/l)	No.	9	8
	Mean ± SE of mean	114 ± 5	127 ± 5
	SD	16	15
Brachial arterial pressure, systolic (mm Hg)	No.	9	6
	Mean ± SE of mean.	161 ± 5	167 ± 6
	SD	16	16
Brachial arterial pressure, diastolic (mm Hg)	No.	9	6
	Mean ± SE of mean.	88 ± 3	87 ± 5
	SD	9	13
Brachial arterial pressure, mean (mm Hg)	No.	9	4
	Mean ± SE of mean	111 ± 3	119 ± 6
	SD	8	18
Resistance (units)	No.	9	8
	Mean ± SE of mean	10.5 ± 0.6	12.1 ± 0.8
	SD	1.7	2.2

Discussion

At rest no difference was recorded between the normal men and the patients of group I. This is in accordance with the results of Chapman et al. (2) and is what might be expected when differences might be small.

Low cardiac output and high arteriovenous oxygen difference has been shown

Table VI Comparison of the calculated increase of heart rate cardiac output and arterial pressures at an oxygen consumption increase of 100 ml/min in normal old men and patients with unlimited working capacity after a myocardial infarction

		Normal persons	Myocardial infarction. Unlimited working capacity
Heart rate increase	No.	14	13
	Mean ± SE of mean	5.1 ± 0.4	6.5 ± 0.4
	SD	1.7	1.5
	Probabil. of diff.	< 0.05	
Cardiac output increase	No.	14	13
	Mean ± SE of mean	0.56±0.04	0.50±0.02
	SD	0.14	0.09
Brachial arterial pressure rise, systolic	No.	14	11
	Mean ± SE of mean	2.6 ± 0.3	4.0 ± 0.4
	SD	1.3	1.4
	Probabil. of diff.	< 0.02	
Brachial arterial pressure rise, diastolic	No.	14	11
	Mean ± SE of mean	0.7 ± 0.2	1.5 ± 0.2
	SD	0.7	0.8
	Probabil. of diff.	< 0.05	
Brachial arterial pressure rise, mean	No	14	11
	Mean ± SE of mean	1.5 ± 0.2	2.5 ± 0.4
	SD	0.8	1.3
	Probabil. of diff.	< 0.05	
Oxygen consumption increase used for calculation	No.	14	13
	Mean	1 040	860
	Range	430–1 491	527 1 148

by Lewis et al. (6) Varnauskas (16) and Müller and Rörvik (10) in patients with left ventricular failure The patients of group II had lower cardiac output and higher arteriovenous oxygen difference than the normal men and the patients of group I and lower stroke volume than the normal men. These findings in group II are suggestive of latent heart failure Poor physical condition following myocardial infarction cannot be completely excluded as a cause of these findings. Inability to return to work was, however in most patients combined with clinical signs of left heart failure and in some with angina of effort.

The patients in group II had higher resistance than the normals and the patients of group I This may be a sign of previous or latent hypertension. Hypertension strains the left ventricle, which together with compromised coronary

circulation made the myocardium fail. On the other hand, increased peripheral vascular resistance is also consistent with cardiac failure as such.

During work differences appeared between the normal men and those patients who had been able to return to work. The increase in heart rate during work was more pronounced in the patients and, unlike the normals, they did not increase their stroke volume during work. All of them did, however increase their cardiac output during work to nearly the same extent as did the normal men owing to their greater heart rates. The increases of the brachial arterial systolic, diastolic and mean pressures were also greater in the patients.

Lewis et al. (6) found a similar failure to increase the stroke volume or a fall in stroke volume during work in 8 of 9 patients in left heart failure with b-normal pulmonary capillary pressure at rest.

This stroke volume difference in response to exercise between normals and patients may thus be regarded as a sign of heart failure in patients. It is the sole haemodynamic abnormality detected in those patients who retained their work after myocardial infarction. It cannot, however be excluded that poor physical condition after the infarction may have influenced the stroke volume.

The patients higher heart rate during exercise may be an indication of diminished working capacity perhaps on account of myocardial failure.

Hypertension is looked upon as a disease predisposing to myocardial infarction and it is known that blood pressure in hypertension may be appreciably lower after an infarction. A more marked blood pressure increase during exercise in patients than in normals discloses a higher peripheral vascular resistance in patients. This is consistent with findings in hypertensive patients Varnauskas (16) and Sannerstedt (11) It remains to be explained, however whether this higher exercise resistance is due to underlying hypertension or also may be the result of heart failure in these patients.

In conclusion, graded haemodynamic differences exist between normal subjects and patients who did and did not return to their previous work (*fig* 1) It is suggested that the cause of these differences might be more or less pronounced myocardial failure in combination with poor physical training following infarction.

Summary

Circulatory data, at rest and during work, for 16 patients who retained their work after a myocardial infarction and data at rest for 8 patients with limited working capacity have been compared with the data for normal men of similar age. These patients who were unable to work had a lower cardiac output and stroke volume and higher arteriovenous oxygen difference than the others. During work differences appeared between the normal men and those patients who retained their work. The latter had a higher heart rate and brachial arterial pressure increase during work and did not increase their stroke volume

Acknowledgement

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Immunologic Studies of Intrinsic Factor

The Reactions of Experimentally Produced Antisera to Human and Hog Intrinsic Factor and of Sera from Pernicious Anemia Patients[1]

By

Ragnhild Gullberg and Sten Kistner

During the past few years many reports have confirmed the development of resistance to therapy in patients with pernicious anemia (p.a.) given hog intrinsic factor (IF) orally (literature reviewed in (19)). Sera from such patients have been shown to prevent the absorption of vitamin B_{12}, when administered orally together with IF to non-resistant cases of p.a. (20). Similar results have also been obtained with sera from untreated or parenterally treated patients (23), although in these cases the inhibitory effect seems to appear less often (21). There was an early suggestion that the development of resistance might be due to an immune mechanism (22) and the serologic findings seemed to support this theory. Results with sera from non-resistant patients make the immunologic concept more obscure but the explanation has been offered that different types of antibodies develop in resistant and non-resistant patients (19). In the latter cases, an autoimmunization has been postulated (23) whereas in the resistant cases the antibody response might be caused by the administration of heterologous IF (20). Circulating antibodies reacting with human or hog IF have recently been demonstrated in p.a. patients by in vitro techniques (11, 12, 21, 24).

The development of an immune response to ingested heterologous IF strongly suggests that IF is absorbed through the intestinal mucosa. It has been suggested that IF like vitamin B_{12} (3, 16) may have an enterohepatic circulation and that the absorbed IF may possess a function in the metabolism of vitamin B_{12}. This is indicated for instance by the different plasma clearances of absorbed and in-

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after leaving the dried agar gel for 12 hours on a Kodak Kodirex X-ray film, one precipitin line containing radioactivity was clearly visible. Prolonging the exposure time to two days resulted in the appearance of one more line and, after 30 days, additional lines were seen. The corresponding lines appeared after a shorter exposure time when the ^{58}Co-labeled vitamin B_{12} was used. The precipitate first observed, containing considerably more radioactivity than those appearing later often partially overshadowed these. The importance of the different radioactive precipitin lines will be further commented upon in the discussion. The same pattern was always observed in this kind of experiment and the antigen causing the major radioactive precipitate will in the following be referred to as the major B_{12}-binding antigenic component, whereas the others will be called the minor components. In immune electrophoresis only 2 components were observed, located in the region of the betaglobulin with the minor one nearer the cathode (fig 1)

When hog gastric juice, gall-bladder bile, or liver homogenate reacted with the same antiserum, similar results were obtained in the autoradiograms as when hog IF preparation was the antigen. By simultaneous investigation of these antigens it was found that the major B_{12}-binding antigenic component of all antigens reacted in the same way with the antiserum, i.e. the precipitin lines, marked by the radioactive vitamin showed a complete fusion from one antigen-antibody reaction to the other (fig 2) indicating that identical structures in the B_{12}-binders from the different sources were responsible for the immune reactions (15) Due to the relatively high activity of the major precipitates the minor B_{12}-binding components could not be completely studied but at least one of them reacted in an identical way with the antiserum when either IF preparation, liver homogenate or bile was the antigen. Immune electrophoresis of hog bile showed that the precipitate of the main B_{12}-binding antigenic component was located nearer the cathode than the corresponding component in the Lederle hog IF preparation (fig 3) Hog gastric juice was also compared with the two hog gastric mucosa preparations. The major B_{12}-binding component of all these IF preparations showed the same electrophoretic mobility (fig 4)

Hog serum and kidney homogenate also gave rise to precipitin lines containing radioactive vitamin, when reacting with antiserum against hog IF preparation. No signs of identity with the major B_{12}-binding antigenic component of hog IF preparation were observed.

2. *Human antigens reacting with rabbit antiserum to human IF preparations*

When human gastric juice reacted with antiserum to human IF preparation in microdiffusion experiments the prolonged exposure time sometimes revealed one or two additional lines appearing on the antibody side of the main radioactive precipitate first observed. Although two B_{12}-binding components (1 5 8) were observed in the agar gel electrophoresis of the gastric juice, immune electrophoresis showed only a precipitate corresponding to the slowly migrating B_{12}-binder in the region of the betaglobulin.

With human bile and liver homogenate reactions with the same antiserum sometimes appeared in the autoradiogram, and many protein bands were always visible. The reactions of the radioactive B_{12}-binding components of such antigens

jected B_{12} (17) and by the enhanced uptake of B_{12} in the liver when IF is present (literature reviewed in (9)) A different explanation for the effect of IF on the liver has been offered namely that the IF is taken up by the reticulo-endothelial cells as a foreign material (25)

In the present work attempts have been made to study the occurrence of IF outside the stomach and gut using immunologic techniques to identify B_{12} binding components in different tissues and body fluids of human and hog origin Experiments have also been made to study circulating antibodies to IF in p.a. patients and to compare such antibodies with those induced by immunization of rabbits with IF preparations.

Material and methods

Antigens. Two hog gastric mucosa IF preparations were used, WES 942 (Lederle, Pearl River N Y) and GEA 57 (Gadex, Copenhagen) Human and hog gastric juice was collected after *in situ* neutralization with sodium phosphate buffer pH 7.2 (6, 29) Human gall bladder bile and liver slices were obtained from patients undergoing cholecystectomy for uncomplicated stones in the gall bladder Hepatic bile was collected from patients with bile duct drainage. Serum, gall-bladder bile liver and kidney were obtained from newly killed hogs The fresh tissues were homogenized in saline using a glass homogenizer After centrifugation the B_{12} binding capacity of the supernatant was estimated by a dialysis technique (18) Radioactive vitamin B_{12} was added in a slight excess, according to the binding capacity The vitamin preparations contained ^{60}Co-labeled cyanocobalamine (Merck, Rahway N Y specific activity approximately 1 mC/mg) or ^{58}Co-labeled cyanocobalamine (N V Philips-Duphar Amsterdam maximal specific activity 100 mC/mg) Samples with low radioactivity were concentrated by ultrafiltration, using a collodion membrane. All preparatory procedures were performed at + 4 C.

Antisera. Rabbit antisera to human IF and hog IF (Lederle) were prepared as described before (7) Besides electrophoretically purified human IF (7) human gastric juice concentrated by ultrafiltration was used for immunization. Eight rabbits received IF preparations with or without B_{12} added.

Sera from 20 p.a. patients were studied. Eleven of these were resistant to 25 mg hog IF (GEA 57) orally in the Schilling test. Sera from 20 blood donors were used as controls.

Antigen-antibody reactions were studied with agar gel diffusion technique (14) and immune electrophoresis (2) using autoradiography (Kodak Kodirex, Kodak Crystallex X ray film or Ilford N 40 plates) to visualize B_{12}-containing precipitin lines (4, 7 11). A micro-procedure (28) was used in most agar gel diffusion experiments. To demonstrate antibodies of low concentration in p.a. sera, dilutions of antigen were used, to which vitamin B_{12} of a high specific activity had been added Electrophoretic retention tests (11 12, 24) were performed in agar gel. In such tests the reaction of IF with gamma-globulin in antiserum was demonstrated when IF bound B_{12} remained at the site of application or migrated with the gammaglobulin and not as a betaglobulin, which was the case after mixing with normal serum. After fixation of the separated proteins in the gel with ethanol-acetic acid solution, unbound B_{12} was washed away and autoradiography was performed. The experiments in this study included approximately 75 immune electrophoresis runs and 500 agar gel diffusion plates.

Results

1 *Hog antigens reacting with rabbit antiserum to hog IF preparations*

When antiserum to hog IF preparation reacted with its homologous antigen a precipitin reaction with a B_{12} binding antigen was always easily detected. In a typical experiment with the micro-technique of agar gel diffusion (28) the antigen sample added contained 2 μg of the Lederle hog IF preparation saturated with ^{60}Co-labeled vitamin B_{12} to give a total activity of 0 001 μC. After diffusion for 1—4 days at room temperature protein precipitin bands appeared and

after leaving the dried agar gel for 12 hours on a Kodak Kodirex X-ray film, one precipitin line containing radioactivity was clearly visible. Prolonging the exposure time to two days resulted in the appearance of one more line and, after 30 days, additional lines were seen. The corresponding lines appeared after a shorter exposure time when the ^{58}Co-labeled vitamin B_{12} was used. The precipitate first observed, containing considerably more radioactivity than those appearing later often partially overshadowed these. The importance of the different radioactive precipitin lines will be further commented upon in the discussion. The same pattern was always observed in this kind of experiment and the antigen causing the major radioactive precipitate will in the following be referred to as the major B_{12}-binding antigenic component, whereas the others will be called the minor components. In immune electrophoresis only 2 components were observed, located in the region of the betaglobulin with the minor one nearer the cathode (fig 1)

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Fig 1



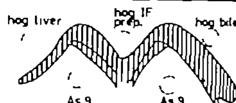


Fig. 2



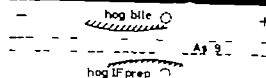


Fig. 3



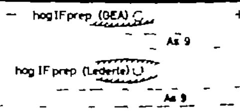


Fig. 4



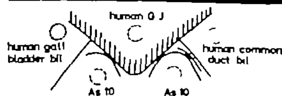


Fig 5



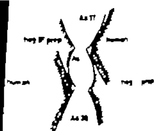


Fig. 6

Figs. 1 to 12 show photographs and schematic drawings f toradiograms f immunodiffusion experiments in 1 % agar gel. Immune lectrophoresis (figures 1 3 4 and 9) was performed in sodium phosphat buffer pH 6.7 μ = 0 05 with 2.5 V/cm for 5 hours. In the Ouchterlony plates (figs. 2, 5 to 8 10 t 12) the gel contained 0.9 N Cl. Radioactiv tamin B_{12} was added t all antigens. The antigens, rabbit antisera and sera from patients with p.a. shown in the figures, are listed below For explanation of the figures, see text.

Antigens Hog IF preparation (Lederle W ES 942 was used when not otherwise marked in the figures), hog gastric juice (G J) gall-bladder bile and liver extract Human gastric juice human gall-bladder bile and common duct bile



Fig. 7



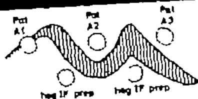


Fig. 8



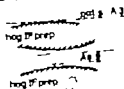


Fig. 9





Fig. 10





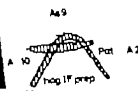


Fig. 11

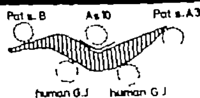


Fig. 12

Rabbit antisera: Antiserum to hog IF preparation, Lederle WES 942 (As 9, As 21), antiserum to the same preparation, to which B_{12} had been added (As 22, As 23). Antiserum to electrophoretically purified human IF B_{12} added (As 10). Antiserum to human gastric juice with (As 17) and without (As 29) B_{12} added.

Sera from p patients. *Pat.s. A* was serum from patient, which contained precipitating antibodies to B_{12} binders in hog IF preparations. Serum *A 1* was collected, hen the oral treatment with hog IF had been withdrawn for year. *A 2* and *A 3* were collected after the patient had received "booster dose" of hog IF orally. *Pat.s. B* was serum from patient, which contained precipitating antibodies reacting with B_{12} binders in human IF preparations.

showed no complete fusion with the reaction of the major B_{12} binding component of the gastric juice (fig 5) When human serum reacted with the antiserum, no evidence of a radioactive precipitate was found

3 *Cross reactions*

A B_{12} binding component in hog IF preparation has been found to react with antiserum to human IF preparation (7) It could now be demonstrated that a B_{12}-binder in human gastric juice also gave a precipitin reaction with some antisera to hog IF preparation When the homologous and heterologous reactions of either antigen were studied in the same experiment it appeared that only minor radioactive precipitates, formed between the antigen and its homologous antiserum, completely fused into the precipitates formed between the antigen and the heterologous antiserum (fig 6 7) All IF antisera were found to cross react with the heterologous IF when investigated by the electrophoretic retention test.

4 *Reactions of sera from pernicious anemia patients*

Nine out of 11 sera from p.a. patients who had become resistant to oral treatment with hog IF caused a radioactive precipitin line when reacting with hog IF preparation. One of these sera also reacted with human gastric juice. Sera from 9 p.a. patients, who had received only parenteral treatment and sera from 20 blood donors did not give precipitates with human or hog IF preparations.

One of the patients with resistance to oral treatment had not received hog IF during the last year Her serum showed a weak reaction with hog IF preparation. She was given the same IF preparation as she had received before (Bendogen) for 10 days, and in a high dose (15 tablets daily) One week later her serum was again investigated The reaction with hog IF preparation was now considerably stronger and the precipitate was situated closer to the antigen basin (fig 8) Serum from this patient did not react with human gastric juice. Immune electrophoresis showed that the reaction between the patient's serum and hog IF preparation occurred in the same place as the reaction between this antigen and antiserum to human IF preparation (fig 9) In both cases the B_{12}-binding antigen appeared somewhat more alkaline than the major B_{12} binding component of hog IF reacting with the homologous antiserum.

Serum from the same patient was compared with rabbit antiserum to hog IF preparation when they both reacted with the antigen in the same diffusion experiment. The radioactive precipitate formed between the patient's serum and hog IF preparation crossed over the major radioactive precipitate formed between this antigen and its rabbit antiserum and completely fused into a minor one, situated on the antibody side of the main line (fig 10 11)

In a similar way human gastric juice was brought to react against the p.a. serum, which gave a radioactive precipitate with this antigen and rabbit antiserum to human IF preparation. The precipitin line formed against the p.a. serum completely fused into the major radioactive precipitate formed between the antigen and its rabbit antiserum (fig 12)

Discussion

It has earlier been discussed whether antiserum obtained after immunization with IF preparations contains antibodies

against IF (7) This assumption is supported by the finding that such antisera inhibit IF activity in vivo (26) and that in immune electrophoresis the reaction with B_{12}-binding antigen occurs in the position of IF in the electropherogram (1 5 8, 10) Immunologic techniques may thus be used to study the IF For its identification in body fluids and tissue homogenates it would have been necessary to compare the reactions of such antigens to those of pure IF Since such a preparation was not available, the present results only inform about B_{12}-binding components in the antigens studied. Their quantitative relationship cannot be determined. The terms "major" and "minor" used in this connection refer to the relative amounts of radioactivity observed in the precipitates, in the absence of a more precise characterization. Nevertheless, some conclusions may be drawn concerning the IF by comparing the immune reactions of B_{12}-binding substances from different tissues and fluids, using experimentally produced antisera to IF preparations and sera obtained from p.a. patients.

Using the microdiffusion technique it was found that the reaction between IF preparations and their homologous antisera resulted in the appearance of several radioactive precipitates. Besides the large easily detectable one, additional weak lines were seen. This seldom occurred when the macrotechnique was used, perhaps due to the smaller volume of reactants added per volume agar gel in those experiments. The very thin gel in the microprocedure gives a better resolution between different precipitates. The results indicate the existence of several B_{12}-binding components in the IF preparations of human and hog origin. The nature of these components is unknown. They may all originate from IF which might in part have been chemically altered during the preparatory or experimental procedure. Since impure preparations were used at least some of them may represent unspecific B_{12}-binders without IF activity

A B_{12}-binding component in hog bile and liver homogenate reacted in the same way as the main B_{12} binding component of hog IF preparations, when tested with antiserum to hog IF (fig 2) B_{12}-binders in hog kidney homogenate and serum also reacted with this antiserum but no reaction of identity was observed when compared with the major reaction of the IF preparation. Preliminary results with antisera to hog liver homogenate and bile confirm the observation of a common antigenic structure in B_{12}-binders of liver bile and gastric juice or mucosa in the hog These results may support the theory concerning the passage of absorbed IF through the liver to the bile (13 17 27) However immune electrophoresis indicated that some difference existed between such B_{12}-binders, since the radioactive antigens in hog bile and IF preparations did not have an identical electrophoretic migration (fig 3) — In comparison, the major radioactive precipitate obtained when human IF preparation or gastric juice reacted with its homologous antisera was not at all influenced by the presence of human bile (fig. 5) or liver homogenate in the same experiment. These findings may possibly imply a different metabolism of IF in man and hog.

Results with sera from p.a. patients, resistant against treatment with hog IF give further support to the theory that the development of resistance is due to an immune mechanism. This was especially emphasized in investigations of serum from the patient who showed an anam-

showed no complete fusion with the reaction of the major B_{12} binding component of the gastric juice (fig 5) When human serum reacted with the antiserum, no evidence of a radioactive precipitate was found

3 *Cross reactions*

A B_{12}-binding component in hog IF preparation has been found to react with antiserum to human IF preparation (7) It could now be demonstrated that a B_{12} binder in human gastric juice also gave a precipitin reaction with some antisera to hog IF preparation. When the homologous and heterologous reactions of either antigen were studied in the same experiment, it appeared that only minor radioactive precipitates, formed between the antigen and its homologous antiserum, completely fused into the precipitates formed between the antigen and the heterologous antiserum (fig 6 7) All IF antisera were found to cross react with the heterologous IF when investigated by the electrophoretic retention test.

4 *Reactions of sera from pernicious anemia patients*

Nine out of 11 sera from p a. patients who had become resistant to oral treatment with hog IF caused a radioactive precipitin line when reacting with hog IF preparation. One of these sera also reacted with human gastric juice. Sera from 9 p.a. patients, who had received only parenteral treatment and sera from 20 blood donors did not give precipitates with human or hog IF preparations.

One of the patients with resistance to oral treatment had not received hog IF during the last year Her serum showed a weak reaction with hog IF preparation. She was given the same IF preparation as she had received before (Bendogen) for 10 days and in a high dose (15 tablets daily) One week later her serum was again investigated. The reaction with hog IF preparation was now considerably stronger and the precipitate was situated closer to the antigen basin (fig 8) Serum from this patient did not react with human gastric juice. Immune electrophoresis showed that the reaction between the patient's serum and hog IF preparation occurred in the same place as the reaction between this antigen and antiserum to human IF preparation (fig 9) In both cases the B_{12}-binding antigen appeared somewhat more alkaline than the major B_{12} binding component of hog IF reacting with the homologous antiserum.

Serum from the same patient was compared with rabbit antiserum to hog IF preparation when they both reacted with the antigen in the same diffusion experiment. The radioactive precipitate formed between the patient's serum and hog IF preparation crossed over the major radioactive precipitate formed between this antigen and its rabbit antiserum and completely fused into a minor one, situated on the antibody side of the main line (fig 10 11)

In a similar way human gastric juice was brought to react against the p.a. serum which gave a radioactive precipitate with this antigen, and rabbit antiserum to human IF preparation. The precipitin line formed against the p.a. serum completely fused into the major radioactive precipitate formed between the antigen and its rabbit antiserum (fig 12)

Discussion

It has earlier been discussed whether antiserum obtained after immunization with IF preparations contains antibodies

reactions occurred between minor B_{12}-binding antigenic components in hog or human IF preparations and antisera to human or hog antigen respectively. Comparison was made with reactions sometimes obtained between pernicious anemia sera and hog or human IF preparations. The results are discussed with reference to the postulated absorption of IF and the genesis of antibodies to homologous and heterologous IF in pernicious anemia sera.

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nestic response i e the antibody concentration appeared to be higher after she had received a large dose of the IF preparation towards which she had earlier shown resistance, and which had not been given to her for a long period of time (fig 8) This observation is in conformity with that of Schwartz, who studied the ability of patient s serum to inhibit IF activity (19) The development of circulating antibodies in the resistant patients indicates that antigenic structures from IF are actually absorbed

The antibody response in the resistant patients showed an individual variability Precipitating antibodies reacting with hog IF preparation could not be demonstrated in all and in one case serum reacted with human gastric juice as well as with the hog antigen

It seemed that minor B_{12} binding antigenic components of IF preparations participitated in the cross reactions with the rabbit antisera to heterologous IF (fig 6 7) The same B_{12}-binders of hog IF preparations seemed responsible for the reactions with sera from the resistant patients (fig 10 11) One might assume that these B_{12} binding components represent breakdown products of IF which have lost species-specific groups, thus being more apt to cross react. The reaction between patient sera and hog IF preparation would then indicate that only broken-down hog IF had reached the antibody producing cells in the patient after being absorbed

Autoantibodies in p.a. patients, reacting with human IF preparations might be produced due to a different mechanism (19) They might be formed against cell constituents which normally do not come into contact with antibody producing cells, but which could reach them under pathological conditions due to leakage from the gastric mucosa. May be the antigen in these cases is represented by a more native form of IF different from the form absorbed in the gut. This could explain why homologous IF given orally promotes the absorption of B_{12} in p.a. patients, even when circulating autoantibodies to IF are present. On the other hand, when p.a. serum containing circulating autoantibodies is given orally together with homologous IF the absorption is prevented (11 21 23) Precipitating antibodies reacting with human IF preparations were found in the serum from one of the p.a. patients. In this case the radioactive precipitate formed between the patient s serum and the antigen completely fused into the major precipitate formed between the antigen and its rabbit antiserum (fig 12)

In sera from p.a. patients different antibodies are thus supposed to be responsible for the precipitin reactions with human and with hog IF This assumption is based upon results obtained with sera from only few patients. The investigation is now extended to include a larger number of p.a. sera.

Summary

The reactions of B_{12}-binding components from different sources with experimentally produced antisera to intrinsic factor (IF) preparations were studied using immunodiffusion technique combined with autoradiography B_{12} binders in hog liver and bile reacted with antisera to hog IF preparation, showing a reaction of identity with the major B_{12}-binding antigenic component of hog IF preparation. Corresponding antigens of human origin reacted with antisera to human IF preparation but not in the same way as the major B_{12} binding component of human IF preparation. Cross

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Cyclic Haemolytic Anaemia Synchronous with Pel Ebstein Fever in a Case of Hodgkin's Disease

By

Poul Rahlov and Aage Videbæk

Anaemia is often an outstanding sign of malignant systemic diseases. In most cases it is due to several causes. In recent years it has been realized — especially by means of methods for determining red cell survival — that hyperhaemolysis is frequently a decisive factor. Even mild hyperhaemolysis may acquire great importance, when the ability of the red bone marrow to compensate for the hyperhaemolysis is seriously impaired. Such a relative bone-marrow insufficiency may be due to the presence of reticulosis in the bone marrow, to treatment with roentgen rays and cytotoxic agents, to the frequent complicating infections, and possibly to malabsorption in the presence of widespread reticulosis in the mucosa of the small intestine. Haemorrhagic diathesis may also be partially responsible for the development of anaemia in patients of this category.

In some instances, the haemolysis is interpretable as auto-immune haemolysis, when red cell sensitization is demonstrable. As a rule, however such sensitization is not demonstrable, although the anaemia and laboratory findings correspond accurately to the findings in Coombs-positive patients. It seems reasonable to believe, therefore, that immune processes are operative in these cases too (7, 8).

Therefore, the elucidation of the pathogenesis of anaemia in such a patient requires quite thorough clinical and laboratory studies.

A manifestation of haemolytic anaemia like that observed in the present case of Hodgkin's disease does not appear to have been reported previously. The anaemia, which was obviously haemolytic, was of a rather peculiar nature, as the haemolysis was cyclic, occurring synchronously with cyclic fever of the Pel-Ebstein type.

Case report

A young man, born in 1941, died in 1962. History of uncomplicated poliomyelitis at the age of one year and said to have had infectious mononucleosis at 16 years.

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In Nov 1959 the patient was referred to the Radium Centre with lymphomas on the neck which had been increasing in size for the past 3 months. No other symptoms, and general condition completely unaffected. Hb 16.1 g/100 ml, ESR 2 mm, RBC 4.90 mill., WBC 6,200. Platelets 200,000. Toxoplasmosis reaction negative. Paul-Bunnel reaction negative. Sternal bone marrow showed normal differential count. Biopsy of a cervical lymphoma revealed changes compatible with the diagnosis of Hodgkin's disease. The lymphomas yielded to local irradiation, total of 1,550 r in the course of 3 weeks.

Fourteen months later cervical lymphomas re-appeared, and again yielded to irradiation, this time 1,350 in 16 days. On continued out-patient follow-up he remained in good health, with normal Hb and ESR, during the subsequent 10 months.

In Oct. 1961 he was admitted to a hospital in Germany with fever and lymphomas. *Microscopic examination of a cervical lymph* node revealed classical Hodgkin disease. Treated for a couple of weeks with cyclophosphamide and prednisolone. Presented himself at the Radium Centre in Nov 1961. At that time his Hb and ESR were again normal.

Two weeks later he was re-admitted after having been running temperature of 40—41 C for a few days, accompanied by fatigue and sweating. On admission his temperature was 40°.5 C, ESR 112 mm, Hb 10.5 g/100 ml, RBC 3.45 mill. WBC 7,000, platelets 190,000. Cold agglutinin titre normal. Widal reaction negative. Blood culture. No growth. During the tw weeks in hospital the temperature spontaneously returned to normal (cf. fig. 1) the ESR fell to 41 mm, the Hb rose to 11.3 g/100 ml. N chemotherapy was administered. A this time no lymphomas were present, neither peripheral nor mediastinal.

H remained well at home for two weeks, after which he became hyperpyretic again. On re-admission his temperature was 39° 1 C, but his general condition was not affected. Moderate generalized lymphomas. The spleen was palpable about 1 cm below the costal border and there was mild scleral jaundice. Hb 8.3 g/100 ml, falling in 6 days to 6.8 g/100 ml, reticulocytes 14‰ platelets 285,000, WBC 6,000 with 7 % lymphocytes. Serum bilirubin 2.2 mg/100 ml, urine bile-stained, faeces of normal colour. Repeated blood cultures yielded no growth of bacteria. Cold agglutinin titre 0. Widal and leptospirosis reactions normal. Malaria parasites could not be demonstrated. Repeated investigations for blood in the faeces gave negative results, and there was no haematuria. Coombs direct reaction was negative, and the fragility of the red cells normal (0.48—0.34 % NaCl). The sternal marrow showed maximum hyperplasia with 33 % normoblasts. Chest radiography revealed moderate enlargement of the hilar lymph nodes. Paper electrophoresis showed reduced albumin, slightly elevated α_2-globulin, but no other abnormalities.

During the further course, Pel-Ebstein fever with hyperpyrexia for bout 2 weeks alternated with afebrile periods of about 3 weeks. Fig. 1 shows that synchronously with the febrile periods the patient had a highly elevated ESR and a haemolytic *syndrome which disappeared during the* afebrile intervals. These phenomena repeated themselves 4 or 5 times during the 5 1/2 months in hospital, although during the last 6 or 7 weeks before death they were somewhat masked by haemorrhages and by the treatment with transfusions, cytostatics and high doses of prednisone which had been instituted at that time. *During these weeks the patient* went steadily downhill and died in a state of refractory anaemia, fever and cachexia.

Autopsy (Dr Johannes Clemmesen) revealed lymphogranulomatous lymph nodes in the mediastinum and retroperitoneal space as well as multiple foci in the spleen, liver and spine. There was moderat enlargement of the liver as well as the spleen. Microscopic examination revealed Hodgkin disease. Moreover widespread bronchopneumonias and bilateral hydrothorax.

During an afebrile interval in March 1962 the red cell survival, determined by Cr^{51} labelling of the patient's own red cells, was found to be normal (26 day) (Dr I Ebbesen, Central Laboratory)

Discussion

The present case of Hodgkin's disease confirmed by biopsy and autopsy was closely followed for 2 1/2 years. In

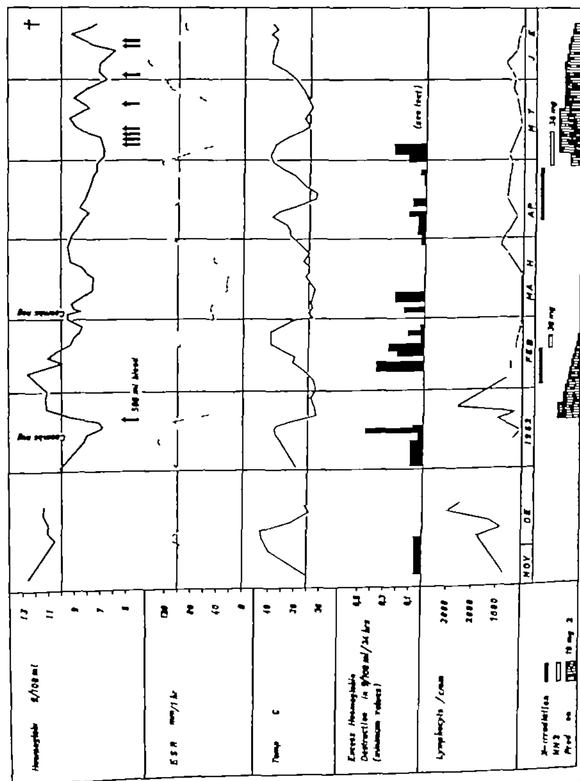


Fig 1 Graphic representation of cyclic changes in temperature anaemia, erythrocyte sedimentation rate, and lymphocyte counts in a patient with Hodgkin's disease. The figure also shows blood transfusions, treatment with prednisone, roentgen, and cytotoxics. Furthermore, the periodical excess destruction f red cells is shown.

often — as in the present case — without demonstrable sensitization of the red cells (2, 8). Haemolytic anaemia does not appear to have been described previously as a regularly recurring phenomenon or to have ever been related to "periodic disease". However, in the present case of Hodgkin's disease with characteristic Pel-Ebstein fever during 6 months, haemolytic anaemia was found, but strangely enough only during the febrile periods, while during the afebrile phases it disappeared.

Pel-Ebstein fever is characteristically a recurring symptom, a kind of "periodic disease" in 5—7 % of all patients with Hodgkin's disease (1, 6). It may be assumed that it is the periodic fever which gives rise to the periodical haemolysis, as elevation of temperature above normal may in some cases promote haemolytic activity (4). Since, however, haemolysis was not increased during the afebrile intervals, it is perhaps more reasonable to assume that the cause of the haemolysis was only periodically present, possibly in the form of periodically occurring antibodies which were perhaps also responsible for the fever setting in during the same period, for the greatly elevated sedimentation rate and for the marked lymphocytopenia.

This brings to mind the case reported by Stacher and Böhnel (5) of cyclic lymphocytopenia in a 63-year-old man with chronic lymphogenous leukaemia. At intervals of 3—10 days this patient had attacks of chills, fever up to about 40° C, and polyuria of 12—14 hours' duration. At the same time the lymphocyte count dropped from 150,000—200 000 to about 5,000/μl, only to rise again, at the end of a day or two, to the initial values. During these paroxysms, but not at other times, the authors could demonstrate a lymphocyte-destroying factor active against the patient's own as well as donor lymphocytes.

Our patient did not, like some patients with Hodgkin's disease and haemolytic anaemia, have an increased serum concentration of immunoglobulins, plasmocytosis, a false positive Wassermann reaction, iso-agglutinins, etc. Despite a normal electrophoretic pattern, a formation of antibodies might still have been responsible for the haematological change (cf. the frequent finding of e. g. red-cell adsorbable antibodies in immunoparetic patients with, e. g., chronic lymphogenous leukaemia).

The discussion on the occurrence of these antibodies in patients with malignant reticulosis has been revived by Kaplan and Smithers (3) hypothesis that the malignant reticulum cells behave as transplanted antibody producing cells. These cells are then supposed to oust the immune apparatus of the host, the patient succumbing in a state dominated by autoimmune haemolytic anaemia, lymphocytopenia and cachexia, corresponding to "runt disease".

The pathogenesis of "periodic disease" is still a complete mystery. Exogenous causes must be considered, for instance cytotoxic therapy or roentgen therapy. However, Pel-Ebstein fever does not presuppose any preceding treatment, and indeed in the present case no treatment had been given immediately before the febrile periods set in.

Recently a case of periodical febrile episodes in a case of toxoplasmosis has been reported (9) for the first time in the literature. It was postulated that these episodes were due to a cycle of reproduction in *T. Gondii* in the lymphatic tissue similar to the *Plasmodium malariae* in the red cells. However, periodic fever has not

particular during the last 8 months, while the course was characterized by fever of the Pel Ebstein cyclic type and synchronous haemolytic anaemia.

From fig 1 it may be seen that through out the observation period the erythrocyte sedimentation rate fluctuated in parallel with the febrile attacks. The sedimenta tion rate reached peak values of 100—140 mm at the same time as the febrile attacks reached a maximum of about 40—41 C while during the afebrile periods the ESR was very low sometimes quite normal.

Fig 1 shows, moreover that especially during the first periods of hyperpyrexia the haemoglobin level distinctly fell as the temperature rose, while it showed a marked increase during afebrile periods. For several reasons, therefore, the period ical anaemia must have been haemolytic.

Haemorrhagic anaemia can be ruled out as haemorrhagic diathesis was not present until the last few months and as repeated microscopic studies of the urine and investigations of the faeces prior to that time had failed to disclose occult bleeding Blood samples were obtained in uniform quantities throughout the observation period and apart from the terminal 2 months only one blood transfusion had been administered The anaemia was normocytic and normochromic, associated with a varying degree of jaundice and dark-stained urine but with normal-coloured faeces The bone marrow erythropoiesis was very brisk, and repeated reticulocyte counts revealed moderately elevated values. Despite the very high ESR, Coombs direct test was normal. The cold agglutinin titres were normal and so was the fragility of the red cells During an afebrile interval the half life of Cr^{51} was normal but unfortunately there was not sufficient Cr^{51} activity left in the blood at the time of onset of the next haemolytic period, 4—5 weeks after the Cr^{51} labelling had been performed.

In the figure the dark columns indicate the computed excess destruction of red cells. Supposing that no production of red cells takes place, haemolysis of normal extent would make the haemoglobin concentration decrease by about 0 8 % in 24 hours. The black columns show how much more the haemoglobin concentration actually did decrease. The values are corrected for the blood transfu sion given during the second febrile period. Since, according to the reticulocyte counts, a certain production of red cells did take place the actual destruc tion was considerably greater than indicated by the columns which definitely represent minimum values and thus demonstrate that during the very periods of increasing temperature there was a marked hyperhaemolysis. — In other words weighty evidence of cyclic haemolysis.

Furthermore, the figure illustrates the variations in the lymphocyte counts in the blood which may best be evaluated during the first two periods of hyper pyrexia. The count was low while the fever was at a maximum and rapidly increased during the afebrile period. Thus, there seems to have been also cyclic lymphocytopenia. After the third febrile period however a severe lymphocytopenia persisted, but this is only a reasonable consequence of the barely concluded treatment with irradiation and nitrogen mustard There was no regular variation in the granulocyte and platelet counts during the observation period.

Hodgkin s disease is not infrequently combined with haemolytic anaemia but

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The Insulin like Activity in Serum Determined by the Rat Epididymal Fat Method

IV Anti-insulin Inhibition of Insulin-like Activity in Electrophoretically Separated Serum Protein Fractions

By

Jens Lyngsøe

Examination of electrophoretically separated serum protein fractions for insulin-like activity (ILA) using the rat diaphragm method has demonstrated the presence of ILA associated with albumin α_1-globulin and with β-γ-globulin (3 17). Using the rat epididymal fat method, ILA was demonstrated in all serum protein fractions, but the activity showed a distribution with two maxima which were located in the same two protein fractions where ILA can be demonstrated by the rat diaphragm method (10).

When serum to which I^{131}-labelled insulin has been added undergoes electrophoresis as shown by several investigators the insulin migrates with the albumin-α_1-globulin (1 4 12). As result of their studies Randle and Taylor have put forward the hypothesis that the insulin in albumin-α_1-globulin is present in an active non-protein-bound state, while the insulin in β-γ-globulin is present in an inactive, protein-bound state.

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In the present investigation an attempt is made to elucidate the relationship between immunologically active and immunologically inactive insulin in electrophoretically separated serum protein fractions, by determining the insulin-like activity of the fractions, both with the addition and without the addition of anti-insulin. As Gjedde (7) has shown that the insulin like activity of serum is considerably increased by dialysis against running water at room temperature, the protein fractions have been examined both with and without this treatment.

Material and methods

Normal subjects were selected among patients hospitalized for diseases not accompanied by elevated blood sugar or by glycosuria. They had no symptoms of diabetes mellitus, and examination of the 24-hour urine showed no glycosuria.

These normal subjects were examined fasting between 7 a.m. and 8 a.m., 8—10 hours after their last meal. Blood was taken from an

been described previously in toxoplasmosis.

It is possible to synchronize the growth of certain unicellular organisms such as the protozoon *Tetrahymena pyriformis* the unicellular alga *Chlorella* and certain bacteria. By exposing such populations to heat in the form of repeated "temperature shocks light, alterations in oxygen tension or administration of antimetabolites the phases of division may be synchronized If the inhibition is abolished the metabolism and processes of division may be seen to occur cyclically and simultaneously (11 12) *In vitro* too it has been possible to induce an almost complete synchronism in a population of HeLa cells from a human cervical carcinoma after cooling the culture to 4 C for one hour (10)

Hypothetically it might be imagined that the neoplastic reticuloendothelial cells in Hodgkin s disease might be synchronized in their growth in some way or other leading to periodical production of abnormal antibodies and that the clinical manifestation might be periodic disease"

Summary

A case of Hodgkin s disease in a young man is reported in detail During 6 months the disease manifested itself as Pel Ebstein fever Synchronously with the periodical fever he had cyclic haemolytic anaemia which has not been described previously and judging by all the appearances also cyclic lymphocytopenia.

On the basis of *Zeuthen s studies on* synchronized cell cultures, some hypothetical reflections on the pathogenesis of "periodic disease are advanced

Acknowledgement

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The electrophoresis was done at 4° C, with 15—20 mA for 18—24 hours. The protein fractions were separated as follows. A piece of filter paper was held the length of the block so that its edge dipped into it 2—3 mm. This sucked up some of the fluid in the block, and the paper was then stained with brom-phenol blue. The albumin, α_1- β- and γ-globulin fractions could now be clearly distinguished on the filter paper and the localization of the protein fractions in the block could thereby be established. The block was then cut into 5 sections 1) albumin + α_1-globulin, 2) α_2-globulin, 3) β-globulin, 4) a rapid γ-globulin fraction, and 5) a slow γ-globulin fraction.

The protein fractions were now eluted with 0.9 % NaCl and dialysed in Visking dialysis tubes of 3.0 cm diameter. Each protein fraction was divided into two portions. One was dialysed first against running water at 20° C for 24 hours, then against 20 % dextran (dextran 20 % has been prepared from Macrodex 6 % (Pharmacia A/S, Copenhagen) by vacuum evaporation at 60° C. Before use it was sterilised at 120° C for 20 minutes) at 16—18° C for 16 hours and finally against Krebs-Ringer bicarbonate buffer at 4° C for 8 hours. The other portion was dialysed against dextran and Krebs-Ringer bicarbonate buffer at 4° C and was not dialysed against running water. At the end of the dialysis the fractions were diluted with Krebs-Ringer bicarbonate buffer to the volume desired. The Krebs-Ringer bicarbonate buffer solution used has the same electrolyte concentration as that used in the standard insulin solutions (8).

After the dialysis, the ILA of the protein fractions was examined, both with and without the addition of anti-insulin. The amount of anti-insulin added to each ml protein solution was 50 µl, this amount of anti-insulin containing less than 10 µU ILA. The samples were incubated for ILA determination, one hour after adding the anti-insulin. The anti-insulin was prepared by injecting increasing doses of zinc-protamine insulin (NOVO) into guinea-pigs. After treatment for 3 months, and 14 days after the last injection, the blood was tapped by heart puncture. The anti-insulin effect of the serum was then measured by means of the rat epididymal fat method. The serum was used only if one ml could inhibit the effect of more than 25 milliunits of human insulin on the rat epididymal fat.

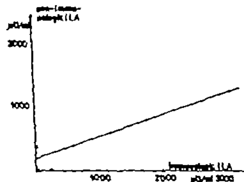


Fig. 1 Fraction 1 Relation between values of immunologic and non-immunologic ILA.
$b_y = 0.313$ $t = 3.73$; $p < 0.01$

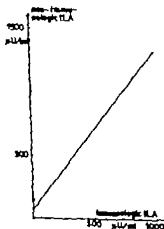


Fig. 2. Fraction 3 + 4. Relation between values of immunologic and non-immunologic ILA.
$b_y = 1.323$; $t = 4.26$; $p < 0.001$

Results

The investigation was carried out on all protein fractions, but here an account will be given only of the findings for the fractions where ILA maximum was found in previous studies (10) namely fraction 1 (albumin + α_1-globulin) with "A peak" and fraction 3 + 4 (β-globulin and rapidly migrating γ-globulin) with "B peak".

Table I. Insulin-like activity and insulin activity in serum protein fractions

Pat. no.	Assay no.	Lambda	Dilution (%)	+ Dialysis at 20 C			— Dialysis at 20 C		
				ILA (μU/ml)	Non-immunological ILA (μU/ml)	Immunological ILA (μU/ml)	ILA (μU/ml)	Non-immunological ILA (μU/ml)	Immunological ILA (μU/ml)
Fraction 1									
271	838	0.32	20.0	3,875	1,325	2,550	750	245	505
273	847	0.33	13.8	4 133	942	3 191	232	0	232
274	853	0.19	8.0	900	525	375	0	0	0
275	857	0.18	7 7	871	702	169	208	195	13
277	869	0.20	7 1	1,890	784	1 106	1 022	284	728
Mean value fraction 1				2,334	—	1 478	442	—	296
Fraction 3									
273	849	0.26	17.0	941	453	488	559	206	353
274	855	0.18	8.0	1 688	1 000	688	363	188	175
275	859	0.10	9.5	1 785	1 155	630	1,890	1,260	630
276	866	0.18	7 1	2,100	1,344	756	952	350	602
277	871	0.21	9.5	441	242	199	294	105	189
Mean value fraction 3				1,391	—	552	812	—	390
Fraction 4									
271	840	0.25	20.0	1 400	925	475	850	650	200
273	856	0.14	10.0	1 400	630	770	140	140	0
275	861	0.22	7 7	1 716	1 196	520	1,365	858	507
276	867	0.24	10.0	660	230	430	260	220	40
Mean value fraction 4				1,294	—	549	634	—	187
Mean value fraction 3 + 4				1 348	—	551	741	—	300

Concentration of protein fraction as percentage of the concentration in unfractionated serum.

arm vein, applying slight stasis. Serum was prepared at 4 C as in previous studies (9). Insulin-like activity (ILA) of the serum protein fractions separated by electrophoresis was determined by a modification of the rat epididymal fat method (8).

Serum electrophoresis was carried out in a polyvinyl chloride block (5). The polyvinyl chloride (Pevikon C 870 Fosfatbolaget, Stockholm, Sweden) was washed repeatedly with water and then with barbiturate buffer at pH 8.6 μ 0 1. The suspended Pevikon was then transferred to a mould, and after sedimentation the surplus buffer was removed by means of filter paper.

In the present investigation, Pevikon blocks measuring 9 7 × 40 0 × 1.5 cm were used. A narrow slot was made across the block about 7 cm from the cathode. Two ml of serum was pipetted into this slot, the sides of which were then pressed together. Excess fluid was sucked up by filter paper. A sponge was placed at each end of the block to form a connection between block and electrode vessels, and the block was then packed in plastic foil and placed between two sheets of glass.

The electrophoresis was done at 4 C, with 15—20 mA for 16—24 hours. The protein fractions were separated as follows. A piece of filter paper was held the length of the block so that its edge dipped into it 2—3 mm. This sucked up some of the fluid in the block, and the paper was then stained with brom-phenol blue. The albumin, α_1- β- and γ-globulin fractions could now be clearly distinguished on the filter paper and the localisation of the protein fractions in the block could thereby be established. The block was then cut into 5 sections 1) albumin + α_1-globulin, 2) α_2-globulin, 3) β-globulin, 4) rapid γ-globulin fraction, and 5) slow γ-globulin fraction.

The protein fractions were now eluted with 0.9 % NaCl and dialysed in Visking dialysis tubes of 3.0 cm diameter. Each protein fraction was divided into two portions. One was dialysed first against running water at 20° C for 24 hours, then against 20 % dextran (dextran 20 % has been prepared from Macrodex 6 % (Pharmacia A/S, Copenhagen) by vacuum evaporation at 60° C. Before use it was sterilized at 120° C for 20 minutes) at 16—18° C for 16 hours and finally against Krebs-Ringer bicarbonate buffer at 4 C for 8 hours. The other portion was dialysed against dextran and Krebs-Ringer bicarbonate buffer at 4 C and was not dialysed against running water. At the end of the dialysis the fractions were diluted with Krebs-Ringer bicarbonate buffer to the volume desired. The Krebs-Ringer bicarbonate buffer solution used has the same electrolyte concentration as that used in the standard insulin solutions (8).

After the dialysis, the ILA of the protein fractions was examined, both with and without the addition of anti-insulin. The amount of anti-insulin added to each ml protein solution was 50 μl, this amount of anti-insulin containing less than 10 μU ILA. The samples were incubated for ILA determination, one hour after adding the anti-insulin. The anti-insulin was prepared by injecting increasing doses of zinc-protamine insulin (NOVO) into guinea-pigs. After treatment for 3 months, and 14 days after the last injection, the blood was tapped by heart puncture. The anti-insulin effect of the serum was then measured by means of the rat epididymal fat method. The serum was used only if one ml could inhibit the effect of more than 25 milliunits of human insulin on the rat epididymal fat.

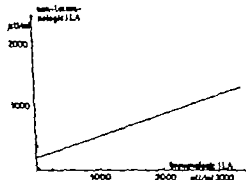


Fig. 1. Fraction 1. Relation between values of immunologic and non-immunologic ILA.
$b_y = 0.313$; $t = 5.75$ $p < 0.01$

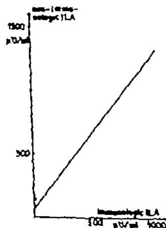


Fig. 2. Fraction 3 + 4. Relation between values of immunologic and non-immunologic ILA.
$b_y = 1.323$; $t = 4.26$; $p < 0.001$

Results

The investigation was carried out on all protein fractions, but here an account will be given only of the findings for the fractions where ILA maximum was found in previous studies (10) namely fraction 1 (albumin + α_1-globulin) with "A peak" and fraction 3 + 4 (β-globulin and rapidly migrating γ-globulin) with "B peak".

Table I Insulin-like activity and insulin activity in serum protein fractions

Pat. no.	Assay no.	Lambda	Dilution (%)	+ Dialysis at 20 C			— Dialysis at 20 C		
				ILA (μU/ml)	Non-immunological ILA (μU/ml)	Immunological ILA (μU/ml)	ILA (μU/ml)	Non-immunological ILA (μU/ml)	Immunological ILA (μU/ml)
Fraction 1									
271	838	0.32	20.0	3,875	1,325	2,550	750	245	505
273	847	0.33	13.8	4 133	942	3 191	232	0	232
274	853	0.19	8.0	900	525	375	0	0	0
275	857	0.18	7 7	871	702	169	208	195	13
277	869	0.20	7 1	1,890	784	1,106	1 022	284	728
Mean value fraction 1				2,334	—	1 478	442	—	296
Fraction 3									
273	849	0.26	17.0	941	453	488	559	206	353
274	855	0.18	8.0	1 688	1 000	688	363	188	175
275	859	0.10	9.5	1 785	1 155	630	1,890	1,260	630
276	866	0.18	7 1	2,100	1,344	756	952	350	602
277	871	0.21	9.5	441	242	199	294	105	189
Mean value fraction 3				1 391	—	552	812	—	390
Fraction 4									
271	840	0.25	20.0	1 400	925	475	850	650	200
273	856	0.14	10.0	1 400	630	770	140	140	0
275	861	0.22	7 7	1 716	1 196	520	1,365	858	507
276	867	0.24	10.0	660	230	430	260	220	40
Mean value fraction 4				1,294	—	549	654	—	187
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Concentration of protein fraction as percentage of the concentration in unfractionated serum.

arm vein, applying slight stasis. Serum was prepared at 4 C as in previous studies (9) Insulin-like activity (ILA) of the serum protein fractions separated by electrophoresis was determined by a modification of the rat epididymal fat method (8)

Serum electrophoresis was carried out in a polyvinyl chloride block (5) The polyvinyl chloride (Pevikon C 870 Fosfatbolaget, Stockholm, Sweden) was washed repeatedly with water and then with barbiturate buffer at pH 8.6 μ 0 1 The suspended Pevikon was then transferred to a mould, and after sedimentation the surplus buffer was removed by means of filter paper

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The electrophoresis was done at 4° C, with 15—20 mA for 18—24 hours. The protein fractions were separated as follows. A piece of filter paper was held the length of the block so that its edge dipped into it 2—3 mm. This sucked up some of the fluid in the block, and the paper was then stained with brom-phenol blue. The albumin, α_1-, β- and γ-globulin fractions could now be clearly distinguished on the filter paper, and the localisation of the protein fractions in the block could thereby be established. The block was then cut into 5 sections 1) albumin + α_1-globulin, 2) α_2-globulin, 3) β-globulin, 4) rapid γ-globulin fraction, and 5) slow γ-globulin fraction.

The protein fractions were now eluted with 0.9 % NaCl and dialysed in Visking dialysis tubes of 3.0 cm diameter. Each protein fraction was divided into two portions. One was dialysed first against running water at 20° C for 24 hours, then against 20 % dextran (dextran 20 % has been prepared from Macrodex 6 % (Pharmacia A/B, Copenhagen) by vacuum evaporation at 60° C. Before use it was sterilised at 120° C for 20 minutes) at 16—18° C for 16 hours and finally against Krebs-Ringer bicarbonate buffer at 4° C for 8 hours. The other portion was dialysed against dextran and Krebs-Ringer bicarbonate buffer at 4° C and was not dialysed against running water. At the end of the dialysis the fractions were diluted with Krebs-Ringer bicarbonate buffer to the volume desired. The Krebs-Ringer bicarbonate buffer solution used has the same electrolyte concentration as that used in the standard insulin solutions (8).

After the dialysis, the ILA of the protein fractions was examined, both with and without the addition of anti-insulin. The amount of anti-insulin added to each ml protein solution was 50 μl, this amount of anti-insulin containing less than 10 μU ILA. The samples were incubated for ILA determination, one hour after adding the anti-insulin. The anti-insulin was prepared by injecting increasing doses of zinc-protamine insulin (NOVO) into guinea-pigs. After treatment for 5 months, and 14 days after the last injection, the blood was tapped by heart puncture. The anti-insulin effect of the serum was then measured by means of the rat epididymal fat method. The serum was used only if one ml could inhibit the effect of more than 25 milliunits of human insulin on the rat epididymal fat.

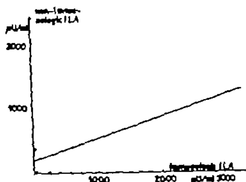


Fig. 1. Fraction 1. Relation between values of immunologic and non-immunologic ILA. $b_y = 0.313$; $t = 3.73$; $p < 0.01$

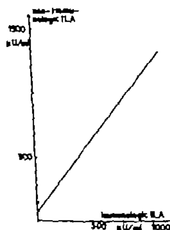


Fig. 2. Fraction 3 + 4. Relation between values of immunologic and non-immunologic ILA. $b_y = 1.323$; $t = 4.26$; $p < 0.001$

Results

The investigation was carried out on all protein fractions, but here an account will be given only of the findings for the fractions where ILA maximum was found in previous studies (10), namely fraction 1 (albumin + α-globulin) with "A peak" and fraction 3 + 4 (β-globulin and rapidly migrating γ-globulin) with "B peak".

The results of determining the ILA of these protein fractions, with and without the addition of anti insulin are shown in table I. The difference has been calculated between the insulin like activity measured with and without the addition of anti insulin This gives a measure of the part of the insulin like activity which is inhibited by anti insulin and is designated "immunological insulin like activity The insulin like activity which is not inhibited by anti insulin is designated "non immunological ILA

Table I shows that in almost all cases the values for the immunological ILA are lower than the ILA values. This means that the anti insulin added has been unable to produce total inhibition of the insulin-like activity of the fractions The relationship between associated values for immunological ILA and "non immunological ILA has been calculated for the protein fractions studied the values for fractions 3 + 4 being calculated together (figs 1 and 2) A dependent relationship between immunological ILA and non-immunological ILA can be demonstrated both for fraction 1 and for fraction 3 + 4 In the case of fraction 1 the regression coefficient is 0 313 and statistically significantly different from 0 ($p<0.01$) For fraction 3 + 4 the regression coefficient is 1 323 and likewise significantly different from 0 ($p<0.001$) The difference between the two regression coefficients is statistically significant ($t = 3.28$, $p<0.01$)

Table I also shows that for all these fractions, both ILA and immunological ILA tend to be higher in the fractions dialysed at 20° C. All determinations of fraction 1 show this, and on the average, both ILA and immunological ILA are seen to increase about 500 % after dialysis at 20 C. Most studies of fraction 3 + 4 show the same tendency and if the values from the two fractions are considered together statistical analysis shows that both ILA and immunological ILA are significantly higher after dialysis at 20 C than without this dialysis (ILA, $p<0.01$ immunological ILA $p<0.02$) On the average, the ILA and the immunological ILA in fraction 3 + 4 increase about 180 % after dialysis at 20° C.

Discussion

Using the rat epididymal fat method other investigators have measured SILA before and after the addition of anti-insulin. These studies have demonstrated that only part of SILA can be inhibited by anti insulin (11 13 15 16) It appeared reasonable, therefore, to assume that in serum there are factors other than insulin with an insulin-like effect on rat epididymal fat, even though SILA when measured by this technique disappears on treatment of the serum with cysteine or glutathione (14 16) An attempt was made in the present study to inhibit the insulin like activity of the serum protein fractions by means of anti-insulin, but complete inhibition of ILA was not found here either However a relationship seemed to exist between the values of ILA that could be inhibited immunologically and the values of the insulin-like activity that could not be inhibited by anti insulin Increasing values of immunological ILA were found to be associated with increasing values of non-immunological ILA. As it appears exceedingly probable that ILA which can be immunologically inhibited can only be due to insulin, the dependence which has been demonstrated suggests that the part of ILA which is not inhibited by anti insulin is likewise caused by insulin

If that part of ILA were due to another substance, it could hardly be expected that its activity in the serum protein fractions would depend on insulin activity.

These results suggest that insulin localized both in albumin-α_1-globulin and in β-γ-globulin is in a form which is immunologically active as well as in a form where the immunological activity is hidden, but that both these forms can show metabolic activity with respect to rat epididymal fat.

In the present study it was found that both ILA and immunological ILA had higher values in protein fractions dialyzed at 20° C than in fractions dialyzed and stored at 4° C. As it is unlikely that a greater destruction of insulin takes place at 4° C than at 20° C, these results appear to indicate that the "warm dialysis" activates some insulin which is without metabolic effect in the untreated protein fractions. This activation occurs both in albumin-α_1-globulin and in β-γ-globulin, that is, corresponding to the A and B peaks of the insulin-like activity.

The cause of the insulin activation found to take place after warm dialysis is unknown. Recent studies strongly suggest, however, that a part of the insulin in serum is bound to protein (2, 17). In this state the insulin has no effect on rat diaphragm. On the other hand, protein-bound insulin appears able to act on rat epididymal fat, as a factor is found in the fatty tissue that can release protein-bound insulin (3). Studies of the ability of the insulin-like activity to diffuse through membranes of varying pore size suggest that only the non-protein-bound insulin is inhibited in its metabolic effect by anti-insulin (6). In the present study, therefore, incomplete inhibition of ILA as found after adding anti-insulin to protein fractions likewise suggests that the rat epididymal fat method records insulin that is bound to protein.

In the present study a rise was found in the immunological ILA after "warm dialysis". This must mean that the warm dialysis has resulted in an increase in the amount of non-protein-bound insulin, and it is reasonable to assume that this increase is the result of a dissociation of insulin from a protein-bound form.

In all the protein fractions examined in the present study both an incomplete inhibition of ILA after addition of anti-insulin and an increase in the immunological ILA after warm dialysis have been found. These results suggest that insulin is found in the protein-bound state both in albumin-α_1-globulin and in β-γ-globulin, these fractions corresponding to the A and B peaks of the insulin-like activity. This need not signify that the protein bond is of the same nature in the two ILA peaks. The present study showed actually that the regression coefficients giving the relationship between immunological and non-immunological ILA differed in the A and B peaks. Moreover after "warm dialysis" a greater percentage increase in ILA and immunological ILA was found in the A peak than in the B peak. These results support the view that the insulin-protein bond in the A peak is different from that in the B peak. Studies are being made to elucidate the biological significance of these findings.

Summary

The present study of the insulin-like activity of serum protein fractions separated by electrophoresis has shown that the addition of anti-insulin to albumin-α_1-globulin and to β-γ-globulin fractions resulted in only partial inhibition of the

insulin like activity in these fractions. In the protein fractions examined a relationship could be demonstrated between immunologically active and immunologically inactive ILA. With increasing values of immunological ILA there were also increasing values of non immunological ILA.

A comparison of the protein fractions dialysed at 4 C and 20 C showed that in all fractions examined there was an increase in both ILA and immunological ILA after dialysis at 20 C.

These results suggest that insulin in protein bound form occurs in both albumin-α_1-globulin and β-γ-globulin

Acknowledgements

Supported by Statens almindelige Videnskabsfond, Denmark.

The insulin was supplied through the courtesy of Dr H C. Hagedorn, M. D Nordisk Insulin Laboratorium, Denmark.

I wish to express my gratitude to Mrs. Tove Dreyer Sorensen for skilful technical assistance.

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Potassium and Magnesium Turnover in Magnesium Deficiency

By

VILLY POSBORG PETERSEN

In a previous paper (11) clinical and physiological findings were reported in a case of magnesium deficiency due to intestinal malabsorption. It was found that magnesium supplement corrected not only the magnesium deficiency but also a calcium deficiency which was caused by abnormal loss of endogenous as well as of dietary calcium. Furthermore, magnesium therapy was able to induce retention of potassium, phosphorus and nitrogen.

In the present paper an extension of these studies is presented. After continuous treatment with magnesium supplement for one year and a half, the patient experienced a relapse of symptoms, which was due to a duodenal ulcer and possibly a flare-up of regional enteritis. These events offered an opportunity to study the spontaneous recurrence of magnesium deficiency and to investigate metabolic balances during treatment with vitamin D and potassium supplement. Also included are compositional and turnover studies of potassium and magnesium before and after renewed correction of the magnesium deficiency

Submitted for publication April 23, 1963.

Methods

Balance study

The patient was placed on a high-protein, low-fat diet containing 920 mg calcium, 1,800 mg phosphorus and 25 mEq magnesium. The intake was determined by analysing one or tw duplicate daily rations in each balance period, which extended over six days. Distilled water was used for drinking purposes. Urine was collected in 24-hour periods, and faeces pooled and analysed in 6-day periods, separated with carmine markers. Food and faeces were homogenized and diluted with distilled water to known volume. Mineral analyses were performed on aliquots after wet ashing with concentrated nitric acid and 70 % perchloric acid. Magnesium in serum, urine and ashed samples was determined by flame photometry (1); otherwise the analytical methods were as previously described (11).

Isotope studies

Potassium[42] and magnesium[28] were obtained from the Radiochemical Centre, Amersham, England. Two studies were carried out with each isotope, one during magnesium deficiency and one several months later after treatment with oral magnesium supplement. After having voided urine the patient was given 100 μc of potassium[42] intravenously as chloride in isotonic solution. Urine was collected t intervals for the next 48 hours and analysed for radioactivity and stable

insulin-like activity in these fractions. In the protein fractions examined a relationship could be demonstrated between immunologically active and immunologically inactive ILA. With increasing values of immunological ILA there were also increasing values of non immunological ILA.

A comparison of the protein fractions dialysed at 4 C and 20 C showed that in all fractions examined there was an increase in both ILA and immunological ILA after dialysis at 20 C.

These results suggest that insulin in protein bound form occurs in both albumin-α_1-globulin and β-γ-globulin.

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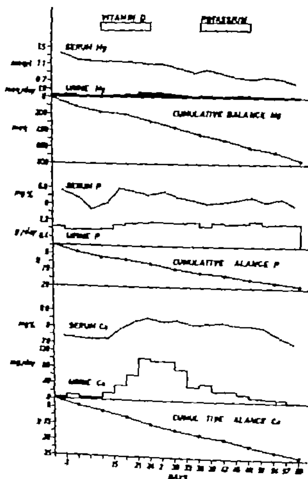


Fig. 1 Magnesium, phosphorus and calcium in serum and urine, and cumulative balances of these elements during 60-day study Vitamin D was administered intramuscularly as an aqueous dispersion of D_2 in doses of 40,000 and 80,000 units in each of two 6-day periods. Potassium supplement was given orally 80 mEq per day

are presented in fig 1 This study extended over 60 days, divided into 10 periods each of six days duration. It was found previously that oral feedings of vitamin D were without effect on calcium, magnesium or phosphorus metabolism in this patient. In the present experiment itamin D was administered intramuscularly as an aqueous dispersion of vitamin D in doses of 40 000 and 80,000 units in each of two balance periods. It appears that vitamin D produced a sustained calcaemic effect and an increase in renal calcium excretion from near zero levels to 100 mg per day The faecal calcium content remained, however higher than the dietary intake and no net gain of calcium occurred. The progressive calcium deficiency suggests that the changes in serum and urine calcium induced with vitamin D were caused not by improved intestinal absorption, but rather by a bone effect leading to increased mobilization of bone minerals.

The gradual decline in serum magnesium was not arrested by vitamin D or potassium supplements. Urinary mag

potassium. The specific activity of a "spot" urine taken after 36 hours was used for calculation of total exchangeable potassium according to the isotope dilution principle. Stools were collected, homogenised and measured for radioactivity after each defaecation. Magnesium[28] 40 μc were administered intravenously as a carrier free aqueous solution of magnesium chloride. Urine was collected every two hours during the first 12 hours, subsequently every 4 hours. Plasma was obtained from heparinised blood samples at 10 minutes, thereafter at 2-hour intervals for 12 hours and 4-hour intervals from 12 to 28 hours after the injection, at which time the activity of plasma samples had decayed to twice the background level. Faecal activity was determined in stools produced after administration of the tracer until the appearance of carmine given 48 hours after the injection. Radioactivity was measured in a well type scintillation counter connected with a Tracerlab Superscaler

Case report

The patient was a 36-year-old male, who for 12 years had suffered from malabsorption due to regional enteritis and extensive intestinal resections. He was first admitted to this department in January 1960 with steatorrhoea associated with hypocalcaemic tetany and magnesium deficiency Treatment with calcium supplement was without any effect, while oral magnesium therapy resulted in correction of magnesium as well as of calcium deficiency relief of tetany disappearance of abdominal cramps and cessation of diarrhoea. The patient continued on a magnesium supplement and remained well for the following 18 months. In October 1961 he experienced loss of appetite, nausea, abdominal pain and vomiting Because of constipation the patient discontinued the magnesium supplement on his own accord one week before readmission In hospital, vomiting and pain subsided gradually after 2—3 weeks. Radiography of the gastro-intestinal tract revealed a duodenal ulcer and signs of extensive inflammatory involvement of the small intestine. As previously stools were bulky and fatty but bowel motions occurred only once daily

The patient was treated with a low-fat, high protein diet and supplements of calcium phosphate, vitamins A, D and C. Vitamin B_{12} had been given at regular intervals during the preceding 2 years and was continued. On admission his serum magnesium was normal, 1.84 mEq/l and urinary magnesium was 2—3 mEq/24 hours. During the following 6 weeks serum magnesium decreased steadily to a level of 1.30 mEq/l, and renal magnesium excretion fell to less than 0.5 mEq per day During the same period serum calcium decreased from 9 0 to 8 0 mg % and urinary calcium excretion from 100 to 20 mg per day The mild hypomagnesaemia and hypocalcaemia were not associated with appearance of any particular clinical signs or symptoms. He was then placed on a constant diet during which a metabolic balance study was carried out. Within the next few weeks serum magnesium decreased further to a level of 0.64 mEq/l. The clinical condition remained unchanged except for increasing volumes of faeces, which became semifluid and amounted to about 1 kg per day simultaneous with more frequent bowel motions. The rate of intestinal transit, as judged from radiography was only 30 minutes, before the barium meal had entered the colon. The patient was regularly questioned for paraesthesias and stiffness of hands and feet, which did not occur The Chvostek and Trousseau signs remained negative. Reflexes and gait were normal and mental disturbances did not appear After conclusion of the pre treatment experimental programme, the patient was again placed on constant oral magnesium therapy 60 mEq daily as magnesium acetate. On this treatment serum magnesium became normal and has remained so. The diarrhoea subsided and stools became formed, but steatorrhoea persisted. During the following 6 months the patient gained 10 kg in body weight.

Results

Mineral metabolism

The concentrations in serum and the urinary excretions of calcium magnesium and phosphorus together with cumulative balances of these elements,

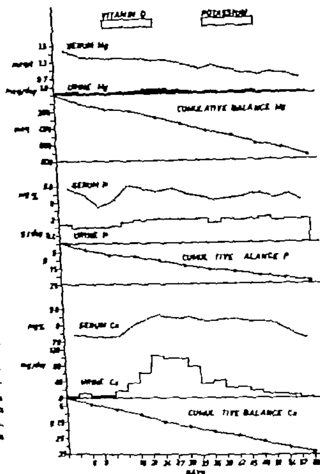


Fig. 1. Magnesium, phosphorus and calcium in serum and urine, and cumulative balances of these elements during 60-day study. Vitamin D was administered intramuscularly as an aqueous dispersion of D_2 in doses of 40,000 and 80,000 units in each of two 6-day periods. Potassium supplement was given orally 80 mEq per day

are presented in fig 1 This study extended over 60 days, divided into 10 periods each of six days duration. It was found previously that oral feedings of vitamin D were without effect on calcium, magnesium or phosphorus metabolism in this patient. In the present experiment vitamin D was administered intramuscularly as an aqueous dispersion of vitamin D_2 in doses of 40 000 and 80,000 units in each of two balance periods. It ppears that vitamin D produced a sustained calcaemic effect and an increase in renal calcium excretion from near zero levels to 100 mg per day The faecal calcium content remained, however higher than the dietary intake and no net gain of calcium occurred. The progressive calcium deficiency suggests that the changes in serum and urine calcium induced with vitamin D were caused not by improved intestinal absorption, but rather by a bone effect leading to increased mobilization of bone minerals.

The gradual decline in serum magnesium was not arrested by vitamin D or potassium supplements. Urinary mag-

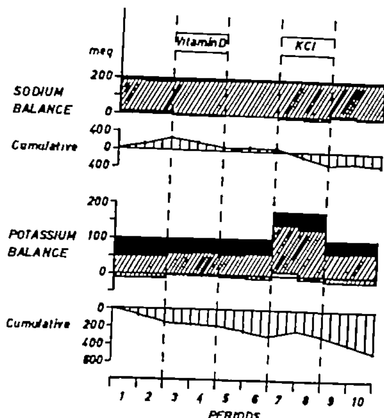


Fig 2. Sodium and potassium balances. Intake is plotted upwards from the zero line, and excretion, faecal (black areas) and urinary (hatched areas) down from the intake line. Cumulative balance is positive above and negative below the zero line

nesium excretion remained at a very low level except when urinary calcium was high which was associated with a small absolute rise in urinary magnesium excretion. This phenomenon, which has been observed repeatedly in other experimental situations (2 10) and also in the present case is probably due to a mutual inhibitory effect of calcium and magnesium on the renal tubular reabsorption of these ions.

Vitamin D administration produced a rise in serum phosphorus and a sustained increase in renal phosphate excretion The phosphaturic effect of large doses of vitamin D is generally ascribed to a renal effect resembling that produced by parathyroid hormone. In subjects deprived of their parathyroids the phosphaturia is associated with falling levels of serum inorganic phosphorus. In the present case serum phosphorus rose after administration of vitamin D and the increased renal excretion of phosphate may at least partly be explained by an increased filtered load of phosphate. Phosphorus balance remained negative, and as with calcium, it is assumed that the excess of phosphorus comes from bone.

Balances of sodium and potassium are presented in fig 2 Sodium metabolism was only slightly influenced by magnesium deficiency with small gains in control periods and slight diuretic responses in periods when calcium and potassium excretions were elevated In contrast, potassium balance showed a persistent loss, and a potassium supplement induced only a small temporary gain, as the greater intake was offset by increased urinary potassium excretion while faecal potassium remained unchanged ranging from 40—60 mEq per day

Potassium turnover

Total exchangeable potassium was measured during magnesium deficiency and after repletion by oral treatment with magnesium acetate for four months. The results which are presented in table I confirm the presence of potassium depletion during magnesium deficiency which as inferred from the present and previous balance studies was caused mainly by a large intestinal loss of potassium. In the present study an attempt was also made to evaluate the loss of endogenous potassium excreted from plasma into the intestinal lumen. For this purpose radioactive potassium in the stools after intravenous administration was measured. In one experiment, performed during magnesium deficiency whil stools were semifluid, radioactivity appeared rapidly in faeces. Stools passed 30 minutes after the injection contained 0.3 % of the injected dose and in the next 48 hours 7 47 % of the dose administered were excreted by the intestinal route. When the experiment was repeated after repletion with magnesium only 0.35 % of the injected dose were excreted with stools passed within 42 hours after administration of the tracer The quantities of endogenous potassium lost with stools were calculated on the assumption that specific activity of potassium excreted into the gut was identical with that excreted into urine within the same period of time. The value of 9 mEq for endogenous potassium as obtained in experiment 2 constitutes 6 % of the total potassium excretion with urine and faeces, which corresponds to the relative faecal output in normal subjects. During magnesium deficiency however the patient lost as much as half of his total potassium output by the intestinal route, and the calculated figure for endogenous potassium should

Table I. Exchangeable potassium and estimation of endogenous faecal potassium during magnesium deficiency (exp. 1) and after magnesium repletion (exp. 2)

	Exp. 1		Exp. 2	
	mEq	mEq/kg	mEq	mEq/kg
Exchangeable potassium	2,060	36	3,270	50
Urine K^{*} (%)	6.74		5.90	
Urine K^{*} (mEq)	124		158	
Faeces K^{*} (%)	7 47		0.35	
Endogenous faecal K^{*} (mEq)	137		9	
Collection period (hrs)	48		42	

be related to the actually determined quantity of stable potassium excreted with faeces in the experimental period, which was 142 mEq The assumption made in calculation of endogenous potassium, that specific activities of endogenous faecal potassium and of urinary potassium are identical, is probably justified, as intravenously injected potassium in animal experiments equilibrates rapidly and simultaneously with kidney and intestine (3) Clearly however the figures for endogenous potassium may be only approximate, due to the possible error induced by the time lag from secretion into the intestinal lumen until recovery of faeces.

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Two isotope studies with magnesium[28] were carried out at eight months interval. The first was done while the patient was in magnesium deficiency four months after oral magnesium supplement was discontinued. The second experiment was performed after repletion by treatment with a mixture containing

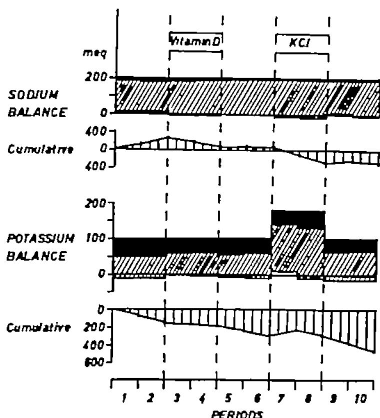


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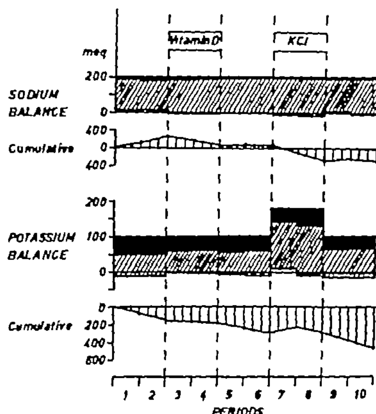


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Table III 24-hour exchangeable magnesium and pool sizes during magnesium deficiency (exp 1) and after repletion with magnesium (exp 2)

	Exp. 1			Exp. 2		
Plasma Mg (mEq/l)	0.78			1.53		
Urine Mg (mEq/day)	0.20			2.78		
Body weight (kg)	61			68		
Magnesium pools	mEq	mEq/kg	% of total	mEq	mEq/kg	% of total
24-hr exchangeable magnesium	115	1.89	–	298	4.45	–
Pool I	14	0.23	12	21	0.31	7
Pool II	46	0.75	40	82	1.21	27
Pool III	55	0.90	48	195	2.96	66

of stable magnesium in the three compartments gave the values shown in table III Total exchangeable magnesium during magnesium deficiency was less than half of that found after repletion, due mainly to a reduction of the slow pool III, which was only 30 % the size of that in the repleted state. This pattern is also reflected in the relative pool sizes, which show that the two rapid compartments accounted for about one-half of the total 24-hour exchangeable magnesium during magnesium deficiency as compared with one-third after repletion.

The renal and intestinal excretion of the magnesium isotope is probably abnormal in both these experiments. In dogs (3) and in human subjects (12) without magnesium deficiency approximately 25 % of the total radioactivity was in the urine within 5 hours after injection, and about 40—50 % was recovered during the first 48 hours. The low renal excretion of isotope as well as of stable magnesium might possibly be due to the patient' total body magnesium not being fully repleted. The faecal excretion of magnesium[28] recovered within 40 hours, was about 3 % of the injected dose. This figure is probably higher than what might be expected in subjects with normal intestinal function, as less than 1 per cent has been found by others (12)

Only few data are available so far with which to compare the present results. MacIntyre et al. (9) reported one study with magnesium[28] in a patient with magnesium deficiency and found values of 2.75 and 2.00 mEq/kg for total exchangeable magnesium and the slow compartment, respectively These authors quote unpublished studies by Caesar and co-workers showing an average value of 4.50 mEq/kg for total exchangeable magnesium in normal human subjects. This figure agrees with that of 4 45 mEq/kg obtained in the present patient after repletion. On the basis of urinary specific activities, Silver et al. (12) obtained values for exchangeable magnesium of a similar order of magnitude in hypertensive patients. By graphical analysis of urinary excretion rates of magnesium[28] these authors found two rapid components

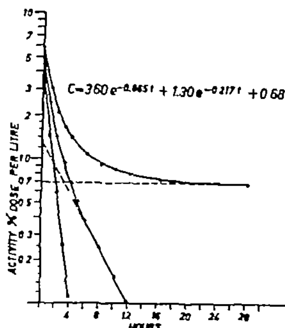


Fig 3 Analysis of plasma magnesium^{28} (C) in terms of exponential components during magnesium deficiency

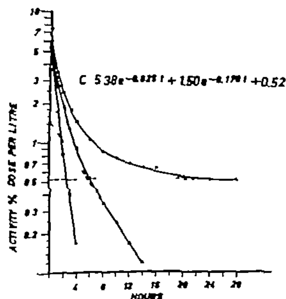


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magnesium acetate, 60 mEq daily taken orally for several months, in consequence of which his serum magnesium concentration ranged between 1 40 and 1 60

Table II. Urinary and faecal excretion of radioactive magnesium

Period (hrs)	Exp. 1		Exp. 2	
	Urine (%)	Faeces (%)	Urine (%)	Faeces (%)
0—24	0.56	0.24	2.49	0.41
25—48	0.49	3.28	1.33	2.62
0—48	1.05	3.52	3.82	3.03

mEq/l (normal range 1 63 mEq/l, S D 0 08) In the time elapsed between the two studies his body weight had increased from 61 to 68 kg

The external loss of tracer within 24 hours after intravenous administration of magnesium^{28} was very low as appears from the figures for urinary and faecal excretion shown in table II. For this reason the plasma activity curves were analysed in terms of a closed multicompartment system (15) in which an apparent equilibrium was established after 24 hours. The experimental data were treated on the assumption that plasma activity is a function of immediate dilution in a central compartment (I) and further transport into two parallel compartments (II III) governed by the rate constants k_1 and k_2. The concentration in the central compartment can then be expressed as

$$C = ae^{-k_1} + be^{-k_2} + c.$$

The plasma activity curves and their components as yielded by graphical analysis are shown in fig 3 and 4 It appears that two rapid exponential components have half lives of about 1 hour and 3 to 4 hours respectively while a third component is a constant which is the final concentration at 24—28 hours. *Calculations of 24-hour ex*changeable magnesium and of the content

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with half lives comparable with those in the present study but their data also indicated a third exponential component with a half life of 14 to 35 hours

It is evident that the presently available short lived magnesium[28] exchanges only with a minor part of total body magnesium Results of whole body analysis (4 16) have shown values of 30—35 mEq/kg of which 60—70 per cent occur in bone. Part of this bone magnesium is exchangeable within 24 hours (3) but the size of this fraction remains unknown

Discussion

Although the concept of the occurrence of several distinct compartments of magnesium in the mammalian body may be an oversimplification of physiological properties, such descriptive categories may still have operational value in the study of magnesium turnover The evidence from the present work is in line with previous studies (9 12) and indicate that the magnesium ion occurs in several pools of varying size and with different turnover rates. A central compartment would comprise extracellular magnesium in which distribution takes place very fast. Exchange with intracellular magnesium probably occurs at rather varying rates in different tissues. In dog experiments Brandt et al. (3) found the highest concentration of magnesium[28] in heart, kidney liver and pancreas and much lower concentrations in skeletal muscle after 24 hours. Assuming that the compartments II and III correspond to magnesium in these organs and skeletal muscle, respectively the present data suggest that in clinical magnesium deficiency muscle magnesium is more depleted than other soft tissue magnesium stores. It would be reasonable to expect that the huge quantities of bone magnesium might be used for buffering soft tissue depletion, as actually occurs in experimental magnesium deficiency in cattle (13 14) Chemical analysis of bone from one patient (9) with magnesium deficiency showed however a normal magnesium content.

The magnesium ion is essential for many enzymic reactions particularly those involving phosphorylated intermediates of carbohydrate metabolism. The relationship between magnesium and other electrolytes, especially potassium which together with magnesium makes up the bulk of intracellular cation is however largely unknown The studies in the present case showed consistently that potassium loss occurred during magnesium deficiency and that a reversal could be induced by magnesium administration High potassium intake was followed by increased renal potassium excretion which indicated that dietary potassium apparently was efficiently absorbed by the intestine, but no lasting effect ensued in terms of potassium balance, which was primarily dependent on an unchanged high intestinal potassium loss. The estimated figure for endogenous potassium loss suggests that perhaps all or at least the greater part of faecal potassium was not unabsorbed dietary potassium, but rather consisted of potassium lost from the extravascular compartment into the intestinal lumen probably into the lower part of the intestinal tract as judged from the rapidity with which the tracer appeared in faeces. The effect of magnesium on potassium transport would then consist, not in any improvement of intestinal potassium absorption but in inhibition of the transport of endogenous potassium into the gut. In a recent paper Heaton

and Parsons (6) reported that potassium retention could be induced also in normal subjects on a high magnesium intake and furthermore that this effect on potassium metabolism was reflected, not in any significant change in renal potassium excretion, but by a 33 to 55 % reduction in faecal potassium output. These authors also showed that a high magnesium intake produced a positive calcium balance in normal subjects by reducing faecal calcium excretion and despite increased urinary calcium excretion, which is similar to what was found in the present case (11).

It seems difficult to explain the effect of magnesium as a non-specific action on intestinal absorption, e. g. due to the slowing-down of intestinal transit time. The magnesium effect is selective in the sense that it concerns mainly potassium and calcium, while the conserving effect on phosphorus (11) may be secondary to that on calcium. Sodium and chloride metabolism was unaffected in normal subjects on high magnesium intake, and in the present case only small and inconsistent changes were found. It is tempting to consider whether the intestinal transport of potassium, by analogy with the renal tubular handling of potassium, includes a proximal absorption and a distal secretion of potassium, which is excreted and that distal secretion is magnesium-dependent. Such a relationship may exist in the kidney (7, 8, 17) as increased loads of filtered magnesium are associated with decreased potassium excretion.

Summary

Studies on mineral metabolism in a case of magnesium deficiency are reported. During spontaneous recurrence of magnesium deficiency negative balances of magnesium calcium, phosphorus and potassium occurred while sodium was only slightly affected. Parenteral administration of vitamin D was associated with a calcaemic effect and increased urinary calcium excretion while negative calcium balance persisted due to a large faecal loss of calcium. Vitamin D did not improve magnesium balance.

Total exchangeable potassium was reduced by one-third during magnesium deficiency as compared with the value obtained after magnesium repletion. An attempt to estimate endogenous potassium loss in stools suggested that large quantities of endogenous potassium were excreted during magnesium deficiency and that high magnesium intake alleviated potassium depletion mainly by reducing faecal output through inhibition of abnormal endogenous potassium secretion into the gut.

Experiments with magnesium^{28} indicated that the magnesium ion occurs in pools differing in size and turnover rate. Total 24-hour exchangeable magnesium was reduced by more than fifty per cent, due mainly to a decrease in the size of a slow pool, which is believed to include skeletal muscle magnesium.

Acknowledgments

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I should like to express my appreciation to Dr. C. B. Madsen for permission to carry out isotope work in the Radiophysics Laboratory. Miss Jytte Sørensen provided careful technical assistance. Vitamin D_3 in aqueous dispersion was kindly supplied by Ferrosan, Copenhagen.

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Royal Academic Hospital, Upsala, Sweden

The Conn-syndrome

Some Diagnostic Aspects

By

Erik Ask-Upmark, Olle Hultén and Folke Knutsson

Suprarenal tumours involving an increased blood pressure are represented by the Cushing-syndrome, the phaeochromocytomas and the Conn-syndrome. With the Cushing-syndrome diffuse hyperplasia of the adrenal cortex is by far more common than real tumours, and the surgical intervention is very much in line with the corresponding treatment in thyrotoxicosis (subtotal resection of the parenchyma involved). Phaeochromocytomas are derived from the adrenal medulla, but may (in some 10 %) be observed outside the adrenal glands, for instance in the organ of Zuckerkandl (= around the origin of art. mesent. inferior) or around the renal artery or in the sinus of the kidney. Quite frequently the history may be characteristic. As to the signs there are two features that should be stressed: the increased urinary excretion of catecholamines and the roentgenological examination (retroperitoneal insufflation of oxygen as well as abdominal aortography: these tumours are well vascularized). Other tests (regitin, etc.) should be avoided as dangerous. The aldosterone-producing tumours, finally, were described for the first time in 1955 by Conn. These tumours are derived from the zona glomerulosa of the adrenal cortex. This zona glomerulosa is responsible for the production of the electrolyte-regulating hormone of the adrenal glands, electrocortine or aldosterone. The main function of this hormone is to stimulate the readsorption of sodium from the renal tubuli, whereas the urinary excretion of potassium is being increased. Hypophysectomy entails atrophy of the adrenal cortex with the exception of zona glomerulosa. In Addison's disease, on the other hand, the zona glomerulosa is involved as well, with ensuing hyperpotassemia and hyponatremia as characteristic features. In Conn's tumour there is on the contrary an increased production of aldosterone with resulting hypopotassemia and hypernatriemia.

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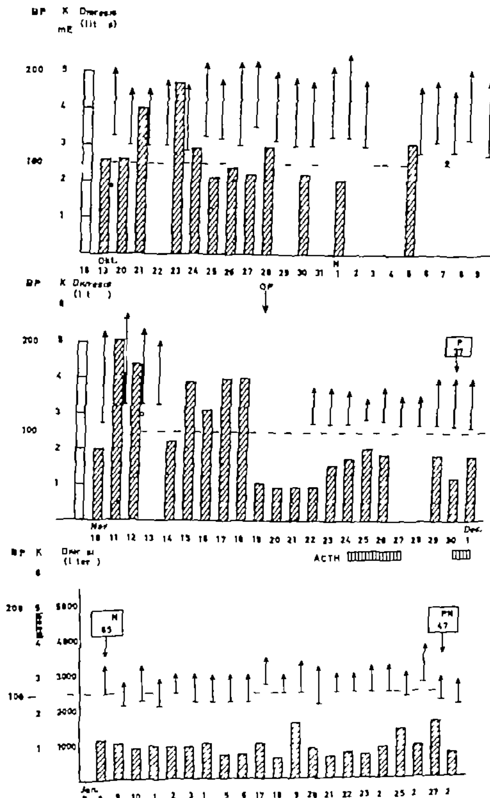


Fig. 1. Blood pressure (arrows from diastolic to systolic). Potassium level (round circles, mEq). Diuresis (striated areas).

It will be seen that following the operation the diuresis decreased, the serum-potassium level increased and the blood pressure dropped, eventually to low-normal levels.

Material

We have had, in the Academic Hospital of Upsala, so far three instances of verified hyperaldosteronism. In one of these three cases the hyperaldosteronism was secondary to an obstruction of the arterial blood supply to one of the kidneys: this case has been reported by Ask Upmark and Lodin (1) and represents a topic referred to by Dollery and coworkers (3) where earlier references are to be had. Another case of severe hypertension in a woman of 48 turned out to be due to a Conn-tumour: this observation has been presented at a meeting here in Upsala by Dr Stina Björk-von Bahr and has been briefly referred to by one of us in another paper (2). The third case runs as follows.

Woman, aged 42. Two children (girls) aged 17 and 14. Since the last delivery hypertension has been observed and she has been treated in various hospitals for this syndrome, lastly in 1955 in our medical department here in Upsala. She was found (in 1955) to present normal levels of catecholamines and 17 ketosteroids in the urine, normal urography but definite nosturia. She was treated with various agents, also ganglion-blocking remedies, but with scanty results. It turned out that she got worse if chlorothiazide was administered: she felt dryness in her mouth, nausea and severe lassitude. She reports that when not taking chlorothiazide thirst, polyuria and lassitude always increased during the 3—4 days preceding each menstruation and also during the days of the menstruation. When admitted Oct. 1962, her general condition was fair. BP 195/125, retinal changes Keith-Wagener II, heart size increased. She had large amounts of urine (on an average 3,000 ml/24 hours but once in a while 4,500 ml) and nosturia was found. Her serum potassium was definitely lowered, being as a rule about 2 mEq/l. The endogenous creatinine was 1.9 mg %. The unfavourable influence of chlorothiazide, the presence of polyuria and nosturia and the low level of serum potassium definitely suggested the presence of hyperaldosteronism. The aldosterone excretion was also found to be considerably increased (104 μg/48 hours as against normal level of 2—17 μg/24 hours)[1]. Retroperitoneal insufflation of oxygen was carried out in our department of roentgenology and revealed a plum-sized enlargement of the right suprarenal gland. Abdominal aortography was also performed: the origins and the course of the renal arteries were normal and it was a striking feature that the suprarenal tumour was not particularly vascularized, in contrast to the findings in phaeochromocytomas.



Fig. 2. Abdominal aortography. Essentially normal conditions.



Fig. 3. Retroperitoneal insufflation of oxygen. The plum-sized tumour of the right suprarenal is easily to be seen.

[1] The determination of the aldosterone level was in this case, as in our other two instances, kindly performed by our colleague Hökfelt of the Karolinska sjukhuset, Stockholm.

The patient was operated upon Nov 19th by Professor Hultén. The suprarenal tumour was found at the expected place, and was large as a big walnut or a small plum. There were no adhesions whatsoever and the tumour was removed together with the rest of the suprarenal gland. On examination the cut surface of the tumour was butter yellow and homogeneous it was well demarcated from the rest of the suprarenal gland, which was normal. There was accordingly no evidence whatsoever suggesting infiltration or malig nancy The tumour is reproduced in fig 4

The postoperative course was smooth. The diuresis dropped immediately to low normal levels, whereas the blood pressure remained elevated for nigh on one week. However six days after the intervention it had been normalized as well.

The patient has since the operation been seen repeatedly in the Department of Medicine by one of us. The blood pressure has remained low-normal and the diuresis is normal. The general condition of the patient is good. The initial increase of her NPN has been relieved to some degree although not completely It has been felt that there is in this case after the operation a certain degree of suprarenal insufficiency It might be wise in the future to confine the removal to the tumour itself, although this must always remain a matter of discretion to be decided by the surgeon only However there may be a slight degree of renal insufficiency as well, as judged from the slight increase of endogenous creatinine. One has to remember that the blood pressure in this case has been allowed for 14 years to stress the arteriolar tissue, not least that of the kidneys.

Comment

The aldosterone-producing tumour of Conn represents the so-called primary hyperaldosteronism, whereas the so-called secondary hyperaldosteronism may be induced either by conditions with edema (such as cirrhosis of the liver nephrosis or cardiac incompetence) or by conditions with malignant hypertension (particularly if this hypertension is caused by impaired arterial blood supply to the kidney (1 3)) A third symptomatic hyperaldosteronism is said to be present in children with alkalosis, retardation of growth and large suprarenals in this type, however there is no hypertension.

The most difficult factor in the diagnosis of a Conn tumour seems to be its rarity although by now more than 100 such instances are said to be known. Phaeochromocytomas are decidedly more common and so is the Cushing-syndrome. Nevertheless, there are two features strongly suggesting the presence of an aldosteronoma the natural history of the hypertension and the roentgenological appearance.

As regards the natural history the Conn tumour is usually encountered in the age between 40 and 60 more commonly in females (M F = 10· 25) The following features are frequently encountered in the history and at the bedside examination.

1 Polydipsia.

2. Polyuria.

3 Isostenuria.

4 Attacks of muscular weakness, for instance when walking upstairs.

5 Increased size of the heart. In females with hypertension the heart size will as a rule remain fairly normal. However if there is an enlarged heart it is a safe guess that the hypertension is caused either by a renal factor or by a suprarenal tumour

6. If chlorothiazide is administered there is apt to be an increase of the symptoms the patients feel a great lassitude, they are subjected to anorexia and nausea.

7 The serum potassium level is lowered, the normal levels ranging between 3 7—4 7 mEq/l.



Fig. 4. The tumour removed. The adjacent suprarenal tissue is to be seen as well.

The patient was operated upon Nov 19th by Professor Hultén. The suprarenal tumour was found at the expected place, and was large as a big walnut or a small plum. There were no adhesions whatsoever and the tumour was removed together with the rest of the suprarenal gland. On examination the cut surface of the tumour was butter yellow and homogeneous It was well demarcated from the rest of the suprarenal gland, which was normal. There was accordingly no evidence whatsoever suggesting infiltration or malignancy The tumour is reproduced in fig 4

The postoperative course was smooth. The diuresis dropped immediately to low normal levels, whereas the blood pressure remained elevated for nigh on one week. However six days after the intervention it had been normalized as well.

The patient has since the operation been seen repeatedly in the Department of Medicine by one of us. The blood pressure has remained low-normal and the diuresis is normal. The general condition of the patient is good The initial increase of her NPN has been relieved to some degree although not completely It has been felt that there is in this case after the operation a certain degree of suprarenal insufficiency It might be wise in the future to confine the removal to the tumour itself, although this must always remain a matter of discretion to be decided by the surgeon only However there may be a slight degree of renal insufficiency as well as judged from the slight increase of endogenous creatinine. One has to remember that the blood pressure in this case has been allowed for 14 years to stress the arteriolar tissue, not least that of the kidneys.

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Fig. 4 The tumour removed. The adjacent suprarenal tissue is to be seen as well

8. The aldosterone excretion in the urine is increased above the normal level which is 2—17 μg/24 hours.

In summary suspicion of a Conn-tumour should be raised if we have to deal with a case of hypertension, particularly in a woman, aged 40—60 if there are polyuria, increased heart size, and attacks of muscular weakness, precipitated or made worse by the administration of chlorothiazide (i. e. chlorothiazide preparations without added potassium). The suspicion approaches a probability if hypopotassemia is to be found and if the aldosterone excretion in the urine is increased.

As to the roentgenological diagnosis it should be remembered that these tumours quite often seem to be rather small, pea-sized or even less, and that in such a case no increased size of the suprarenal shadow is to be expected. Hence, only the positive roentgenology is of deciding value: should it turn out to be negative one must not hesitate in otherwise suspected instances to make a surgical exploration of both suprarenals from behind. However when positive roentgenological evidence is obtained, it will appear as if a rather characteristic feature would be the observation of a suprarenal tumour on retroperitoneal insufflation of oxygen (some 300 such examinations have so far been performed in our department of roentgenology without any incidents whatsoever). On the other hand the ample vascularization of the suprarenal tumour so characteristic for many phaeochromocytomas will not be found in a Conn-tumour. Yet, one cannot refrain from abdominal aortography: on the one hand it will reveal the presence of obstructions of the renal arteries (such as may induce a secondary hyperaldosteronism); on the other hand one may be mistaken in spite of all efforts to the contrary in as much as a phaeochromocytoma may be found. Whereas the phaeochromocytomas belonging to the chromaffin tissue of the adrenal medulla, in 10 % may be found outside the adrenals (from the aortic bifurcation and upwards along the abdominal aorta) the Conn-tumours, being derived from the adrenal cortex, will always be confined to the *suprarenal* gland itself.

We feel that for practical clinical purposes the diagnostic proceedings as outlined above will be sufficient in the vast majority of instances. Much has been written about a differentiation between a primary and a secondary hyperaldosteronism by various tests, such as administration of a large amount of sodium chloride or the introduction of substances establishing a competition with aldosterone (inhibitor-effect) or the determination of the magnesium level in the serum. Whilst such methods may be of considerable interest for laboratory medicine, we feel that they are too circumstantial for the routine work in the clinic and as to the administration of sodium chloride we strongly disfavour such a test in a patient with severe hypertension.

Summary and conclusions

1. A case of primary hyperaldosteronism, caused by a Conn-tumour of the suprarenal cortex, is described: a woman of 42 with hypertension since 14 years.

2. Attention is called to certain characteristic features in the natural history of such a patient, which may strongly suggest the diagnosis, particularly if confirmed by the observation of a

low potassium level in the serum and if possible, by an increased output in the urine of aldosterone.

3 Confirmation of the suspicions may be had either by retroperitoneal insufflation of oxygen as well as by abdominal aortography or by surgical exploration from behind. Attention is called to the differences in the roentgenological appearance between a Conn-tumour and a phaeochromocytoma.

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Elimination of Hypaque (Sodium-3,5-diacetamido-2,4,6-triiodobenzoate) and the Effect of Haemodialysis in Anuria

A Clinical Study and an Experimental Investigation on Rabbits

By

Roy Hansson and Tore Lindholm

Renal damage following X-ray examination with contrast media administered intravascularly has been reported (4 13 19 20)

The use of new intravascular contrast media with less toxic properties (11 12) seems to have reduced the frequency of these complications. Another factor that has undoubtedly contributed to the falling incidence of complications is the increasing knowledge of contra-indications.

Experimental studies (12) have demonstrated a low toxicity of sodium-3,5-diacetamido-2 4,6-triiodobenzoate (hypaque). In morphological studies of the effect on the kidneys of hypaque in currently used concentrations, Berg et al. (6) were not able to demonstrate any histological changes. Hypaque has now been used for several years, but there were no reports of renal complications referable to the use of this contrast medium, until Berlyne and Berlyne (7) reported a case of non-fatal acute renal failure following intravenous urography with hypaque. In their discussion they suggest the possibility that a prolonged high concentration of contrast medium in the blood after intravenous urography can increase the risk of a reaction to the urographic medium when a renal lesion is present. — In one of our cases acute renal failure occurred after the use of hypaque (2)

In one of the 31 patients with anuria after intravascular injection of a contrast medium, who were treated at this clinic, determination of the serum-iodine concentration was made. On the fifth day after the injection of the contrast substance (100 ml of 50 % Umbradil) the concentration of iodine in the serum was 55.2 mg/100 ml (B. Skanse, M. D †)

Submitted for publication April 24 1963.

During dialysis performed to lessen the uraemic intoxication and to remove the retained contrast medium the concentration was reduced to 32 0 mg/100 ml or by 42 % In the following 24 hours, only a slow continuous fall was noted.

In several of the patients treated here because of anuria-oliguria following intravascular injections of contrast media, plain X ray of the abdomen has revealed contrast medium in the intestine (2)

These observations led us to start an investigation into the excretion of intravascularly injected iodine 131 labelled contrast media into anuric subjects. As a contrast medium we chose hypaque, since this and allied derivatives of tri iodobenzoates are predominantly used at present.

A search through the literature failed to give any information on how hypaque is excreted by an anuric subject, although some studies on the distribution of hypaque in kidney-deprived dogs have been made by Langecker et al (16) The main purpose of the present investigation was therefore to study the excretion of hypaque in *bilaterally nephrectomized* rabbits by use of its radioiodinated analogue. If the concentration of hypaque remained high for a long time, the plan was then to investigate whether the elimination could be hastened by extra corporeal dialysis.

Experimental series

In the first two series the plasma-concentrations of intravenously injected contrast medium were determined in normal animals and nephrectomized animals, respectively

In the next two series these experiments were repeated in normal animals and nephrectomized animals respectively but the bile was made to flow through cannulas inserted into the common duct. The concentrations of the contrast medium were determined not only in plasma but also in the bile passed through the cannula. After the end of the experiments *the activity in the removed* intestines and their contents was also measured.

In the fifth series the contrast medium was introduced into the intestine via the common bile-duct and the absorption was studied.

In a further two series the plasma-concentrations were followed in nephrectomized animals. In one of these series the animals were treated by dialysis, and in the other series by extracorporeal circulation without dialysis but otherwise under analogous conditions.

Technique

Rabbits of a mixed breed, males and females, their weights ranging from 2.0 to 2.5 kg, were used. All the operations were made under local anaesthesia (Xylocain® 1 %)

Cannulation of arteries and veins. In all the animals glass cannulas were inserted into one carotid artery and one jugular vein. The operative technique was essentially the same as that described by Alwall et al (1) The technique was simplified, however in that the operation was performed on the morning of the day of the experiment. This was not found to have any disadvantages in the form of bleeding

Nephrectomy For the removal of the kidney the retroperitoneal approach was used and an incision, about 5 cm long was made in the lumbar region parallel to the median line and lateral to the long muscles of the back. The renal stalk was tied en bloc with double silk ligatures. The left kidney was removed at least seven days before and the right kidney immediately before the beginning of the experiment.

Choledochostomy An incision, about 7 cm long was made in the abdominal wall along the median line below the xiphoid process. By blunt dissection the distal 8—10 mm of the common duct were exposed A glass cannula was inserted into the common duct using the same technique as that described for the artery and vein. The glass cannulas designed for the artery proved to be suitable

for the common duct. Where the contrast medium was to be administered through the biliary tract, two similar glass cannulas were inserted, one with the opening distally and one with the opening proximally in the common duct. Tubes connected to the glass cannulas were carried laterally through the abdominal wall by separate incisions. Silk sutures were applied to the abdominal wall.

Dialysis treatment. The combined dialyser ultrafilter described by Alwall (1) was used, but only for dialysis. The arterial and venous cannulas inserted into the vessels of the rabbits were joined to the tubes of the dialyser via cone-shaped glass connectors. The extracorporeal system, which had a capacity of 40 *ml*, was filled with blood from exsanguinated normal rabbits.

Heparinisation. The rabbits were given heparin (heparin® Vitrum) 25 mg, before dialysis was started. During dialysis, which in all cases lasted 4 hours, 5 mg/hour were given. Throughout the dialysis the animals' blood-pressures were checked.

Dialysis fluid. The dialysis fluid was of the same chemical composition as that used for human dialysis in the Medical Clinic B (Renal Clinic) t Lund (2). The total amount of dialysing fluid passing through the apparatus was about 15 l per treatment.

Extracorporeal circulation without dialysis. In these experiments the procedure was the same as in the dialysed series, the only difference being that the animals were connected not to the tubes of the machine but to plastic tube (Portex 18 HE) the length of which was adjusted so that, as in the dialysis series, the extracorporeal system had capacity of 40 ml of blood. The heparinisation and the "treatment time" were the same as in the dialysis series.

Feeding. The rabbits were allowed free intake of standardized vitamin-fortified food in the form of pellets, and free intake of fluid.

Fluid replacement. In the animals which had biliary fistula, fluid was replaced by Ringer' solution, 5.5 % glucose solution, and 1.3 % sodium-bicarbonate solution, according to the volume of the biliary flow.

Collection of urine. A plastic catheter was inserted into the urethra. At the end of each experimental period the bladder was emptied by compression.

Contrast medium. The contrast medium was commercial hypaque (sodium-3,5-diacet amido-2,4,6-triiodobenzoate) labelled with ^{131}I by the method of Liebster et al. (17) (A. B. Leo, Hälsingborg Sweden) and having an initial specific activity of 2.9—4 4 μC/mg in 10—12 % aqueous solution (w/v). The preparation was tested after the completion of an experimental series both by precipitation (orthophosphoric acid to pH 1.0) which showed that less than 5 % of the radioactivity was present in the supernatant after centrifuging, and by chromatography with autoradiograms, which showed the radioactivity to be concentrated in one distinct spot with the same R_F-value as non-radioactive hypaque solution. Precipitation and chromatography were also performed by the method of Liebster et al. (17)

Each rabbit received an injection of about 30 μC ^{131}I corresponding to 11—145 mg of carrier hypaque. The injection was made with a tuberculin syringe weighed with an accuracy of $\pm$ 0.1 mg before and after the injection. A standard was prepared by dilution of weighed solution, as used for injection, with a known volume of 1 % (w/v) sol tion of potassium iodide in water.

Blood samples were taken from the arterial cannula in heparinised test-tubes, about 2 ml in each (5, 10) 15, 30 min. and 1 2, 4 10, and 24 (and 48) hours after the injection. The blood of the animals that were treated by extracorporeal circulation was lso examined 1 2, 3, and 4 hours after the start of the extracorporeal circulation. After centrifugation, 500 μl of plasma were taken with constriction micropipette (Carlsberg type) and diluted with 1 % potassium iodide solution to volume of 3 0 ml in the sample tubes. The same volume was also used for the ^{131}I standard.

To allow of a comparison between the different series the value for the 15-minute sample was in all the series taken as unity and the other values were expressed as a percentage of this value.

Urine samples. According to the anticipated level of radioactivity 50 100, or 1,000 μl were taken from the urine samples and diluted to 3.0 ml in the sample tubes.

Bile samples measuring less than 3.0 ml were diluted to this volume in the sample tubes otherwise 3.0 ml was transferred to the sample tubes.

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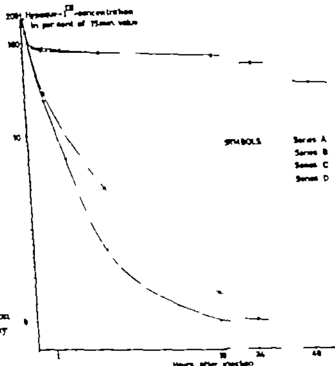


Fig. 1 Effect of nephrectomy upon the plasma-concentration of hypaque.

Series C. Choledochostomy with cannulas in the proximal direction. ¹³¹I-hypaque intravenously. Five rabbits

The plasma-concentrations are shown in table I and fig. 1. The excretion of the contrast medium was somewhat slower in this series than in series A; there are no essential differences between the two series, however. Only with respect to one value (the 4-hour value) is there a significant difference between series A and C [illegible]t the 95 % confidence level ($0.001 < p < 0.01$).

A possible explanation of this difference may be that the kidneys were slightly affected by the choledochostomy (cf. the "hepato-renal syndrome") Unfortunately direct comparisons of renal function could not be made, since no urine samples were collected in series A. The 24-hour urinary excretion of three animals in series C, in which adequate collection of urine could be carried through, amounted to 89 [illegible] 87 and 86 % of the given dose. This urinary excretion agrees with that reported by Schlungbaum and Billion (22) for man (Urografin) and by Langecker et al. (16) for rabbits (Urografin).

Series D. Bilateral nephrectomy. Choledochostomy with a cannula in the proximal direction. ¹³¹I-hypaque intravenously. Five rabbits

The plasma concentrations are shown in table I and figs. 1 and 2. With respect to the plasma-concentrations, this series is essentially consistent with series B.

Table I. Plasma concentrations in series A—D Mean values ± S E of the mean in percentage of the 15-minute values

Series	Time									
	5 min	10 min	15 min	30 min	1 hr	2 hrs	4 hrs	10 hrs	24 hrs	48 hrs
A	175.8 ± 7.3	128.1 ± 7.0	100	57.1 ± 3.5	24.4 ± 2.0	5.7 ± 1.6	0.6 ± 0.09	0.1 ± 0.03	0.3 ± 0.10	—
B	—	—	100	89.7 ± 1.1	82.0 ± 1.3	79.4 ± 1.5	77.0 ± 1.2	64.7 ± 1.6	52.0 ± 2.5	32.4 ± 6.0
C	178.2 ± 11.0	126.3 ± 3.5	100	57.5 ± 1.7	29.1 ± 1.7	11.2 ± 1.4	2.7 ± 0.5	0.2 ± 0.04	0.1 ± 0.04	—
D	—	—	100	89.8 ± 2.5	87.1 ± 0.9	81.9 ± 2.3	76.3 ± 2.3	64.9 ± 1.8	50.7 ± 3.4	—

Excess volumes of urine and bile were measured in a 10-ml volumetric cylinder graduated in 0.1 ml.

The *radioactivity* in the samples was determined in a well-type crystal detector whose photomultiplier tube was connected via a pulse height analyser to a counter (the whole equipment from Tracerlab SC-57 RLI-4SR, and SC-18) On measuring over the peak of 364 KeV 1 μC ^{131}I gave, in the apparatus used, about 2.0×10^{6} c.p.m. with optimal gain and pulse height level against a background activity of 3—4 c.p.m.

Measurements over the removed organs were made with the same pulse height analyser (RLI-4SR) and counter (SC-18) but with a flat type crystal scintillation spectrometer detector (Tracerlab RLD-2) provided with a 17° cone-shaped collimator and lead shielding at a distance of 43 cm from the centre of the sample At this distance 10 μC ^{131}I gave 3.0×10^{4} c.p.m. against a background of 12 c.p.m. The organs were suspended in water after some previous homogenisation. The organ suspension was, at most, 4 cm in depth and was compared with a geometrically equal standard of ^{131}I hypaque suspended in gelatin gel.

Error of the method With the quantity of radioactivity injected and the amounts of plasma over which the activity was measured, the readings had a statistical error which for the single observation at 100 % level averaged 1—2 % and at 1 % level about 10 %.

Results

Series A Intact animals (but, as in all series with arterial and venous cannulas) ^{131}I hypaque intravenously Four rabbits

The plasma concentrations are shown in table I and fig 1 After 4 hours they fell to values below 1 % of the 15-minute value. The animals were followed for up to 24 hours. In these animals the plasma-concentrations were halved in 20 minutes.

Series B Bilateral nephrectomy ^{131}I-hypaque intravenously Seven rabbits

The plasma-concentrations are shown in table I and figs 1 and 2. In this series the plasma-concentrations did not fall to half until after 30 hours and after 48 hours they had fallen to one-third of the 15-minute value.

The kidneys thus play a dominating role in the excretion of Hypaque, but there must be other routes of elimination. To enable an estimation of how much of the substance is excreted via the liver and the bile, the following two series were carried out

ble II. Plasma concentrations in series F—G. Mean values ± S. E. of the mean in rcentage of the 15-minute values

ries	Time									
	15 min	30 min	1 hr	2 hrs dialysis started	3 hrs	4 hrs	5 hrs	6 hrs	10 hrs	24 hrs
F	100	91.4 ± 2.1	86.5 ± 3.7	89.3 ± 4.5	79.3 ± 1.7	77.0 ± 1.2	73.6 ± 2.1	71.8 ± 2.3	66.6 ± 2.3	49.6 ± 1.4
G	100	92.4 ± 1.3	86.3 ± 1.2	81.3 ± 4.1	61.4 ± 1.9	50.2 ± 1.3	42.1 ± 1.4	33.8 ± 1.5	37.1 ± 2.0	27.3 ± 1.8

Series G. Bilateral nephrectomy. ¹³¹I-hypaque intravenously. Dialysis treatment. Stone rabbits

The plasma-concentrations in these two series are shown in table II and fig. 2. It will be seen that ¹³¹I-hypaque is a dialysable substance. From the values at 3 hours and onwards the two series differ with a high level of significance ($0.001 > p$). These observations are confirmed by measurements on the dialysates, in which 47.2 ± 2.4 % of the administered dose was found. At autopsy 24 hours after the injection, 23.4 ± 2.. % of the injected amount of radioactivity appeared in the intestines of the dialysed animals, *as against 45.2 ± 10.5 %* in the non-dialysed group.

In all the animals measurements were made over the thyroid gland, and in no case was any radioactive material found in this organ. In contradistinction to Billion and Schlungbaum (8) we cannot hereby conclude that iodine is not split off from the contrast-medium molecule. *In the injected dose the radioactive* substance forms only a small part, the main part being carrier whose possibly split-off iodine has an equally *great* affinity for the thyroid tissue. The thyroid uptake of any possibly split-off radioactive iodine will therefore be in the same insignificant proportion as that of the amount of radioactive contrast medium to the amount of carrier.

Discussion

The pharmaco-dynamic effect of hypaque has been demonstrated by Lindgren and Törnell (18) and others. These studies, however, have not provided any explanation of the renal lesions following *parenteral* administration of hypaque. Hildreth et al. (10) on the basis of time factors, the size of the dose, and mode of reaction, maintain that the mechanism is probably explained by hypersensitivity. However, they draw *attention* to the limited value of pre-testing in the form of *conjunctival* and intradermal tests. Sobin et al. (23) have observed that intravascular aggregation of erythrocytes occurs after administration of iodine-containing contrast media, and a disturbed blood-flow through the kidneys through this cause is possible. Kutt and Mc Dowell (15) have recently shown that intravascularly administered contrast media in high concentrations bring about a change in the migration pattern of the blood-proteins in electrophoretic studies; this finding could represent a

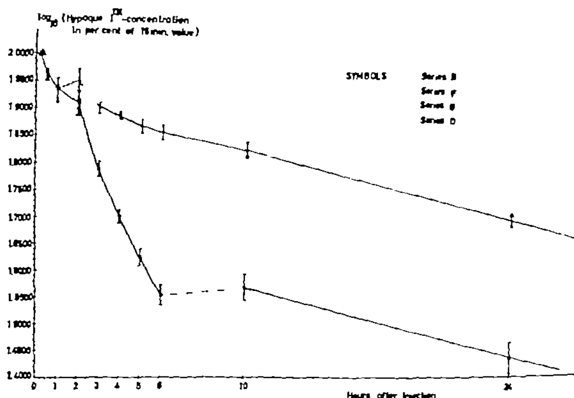


Fig 2. Effect of dialysis upon the plasma-concentration of hypaque in nephrectomized animals.

The factor that constitutes the difference between series A and series C seems to be absent here (possibly the renal function)

In series C the total excretion of the contrast medium in the bile was 0 55 ± 0 14 % of the total administered dose. In series D 24 8 ± 5 4 % of the total administered dose was excreted in the first 24 hours.

In both series C and series D in which the bile-flow was made to pass through a cannula, the activity in the intestine (from the oesophagus to the rectum removed with the contents) was measured at autopsy

In series C 0.21 ± 0 06 % of the given dose were found in the intestine and a negligible amount in the liver In series D 14 0 ± 2.1 % of the given dose appeared in the intestine and 6.7 ± 1 5% in the liver

That the contrast medium which enters the intestine can be re-absorbed is shown in the following series

Series E Choledochostomy with a cannula in both the proximal and the distal direction. ^{131}I hypaque administered to the intestine via the distal cannula. Two rabbits

The blood-concentrations rose in both cases, reaching their highest values in the 2 hour samples. When the rabbits were killed after 24 hours 44 % and 54 respectively of the given dose were found in the removed intestine. Most o the absorbed radioactivity appeared in the urine, whereas control measurements over the liver gallbladder and thyroid showed only minimal amounts (<0.5 /)

Series F Bilateral nephrectomy ^{131}I-hypaque intravenously Extracorporeal circulation without dialysis Six rabbits

damage, it seems that the quickest possible elimination of the substance is desirable.

Our experiments with extracorporeal dialysis show that considerable amounts of the contrast agent can be eliminated by this procedure. Kutt and McDowell (15) have also found that the observed changes in the blood-proteins are reversible by haemodialysis.

Moreover the absorption of the contrast medium in the intestine can probably be reduced by induced diarrhoea. In anuric patients diarrhoea is most suitably induced by oral administration of 100—150 ml of sorbitol in 70 % solution.

an entero-hepatic recirculation mechanism exists.

Experiments in which the artificial kidney was used (dialysis) show that hypaque in vivo is a dialysable substance. Close to half the given dose was removed by 4 hours dialysis.

The development of renal lesions after intravascular injection of contrast media is discussed. The risk possibly attending a prolonged high concentration of the contrast medium in the body is emphasized. The quickest possible elimination of the contrast medium can be achieved by dialysis treatment and also by induced diarrhoea.

Summary

Our observations indicating that contrast media injected intravascularly into anuric patients remain in the body for a long time prompted us to study in rabbits the excretion of sodium 3,5-diacetamido-2,4 6-triiodobenzoate (hypaque)

In intact rabbits the plasma concentrations after intravenous injection fell rapidly with a half-time of 20 minutes. In bilaterally nephrectomized rabbits the half-time was 30 hours. In otherwise intact animals the total excretion of contrast medium in the bile was less than 1 % of the given dose, whereas in bilaterally nephrectomized animals 24.8 ± 5 4 % was excreted in the bile in the first 24 hours. In the latter animals an extrahepatic excretion to the intestine, amounting to 14 0 ± 2.1 % in the first 24 hours, was also demonstrated. By administering hypaque to the intestine via the common duct it could also be shown that the contrast medium is absorbed from the intestine. Accordingly

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change in the properties of the plasma proteins *in vivo* and be a factor concerned in the causation of renal lesions. These high concentrations are however attained only in the vessel that receives the infusion that is, in most cases the cephalic vein. Intravenous urography in patients with myelomatosis, in whom a disturbance of the blood proteins is already present, has been found to be attended with great risks of renal damage (2, 5a 21)

Evidently there is as yet no explanation of the renal lesions that can occur after intravascular administration of contrast media, but it is known that myelomatosis and impaired renal function, shock and infection (2 5 20) can predipose to acute renal failure. Furthermore, it seems that repeated injections of contrast media could increase the risk of renal damage (4 14)

Although the renal damage seems to occur in direct association with the administration of contrast media, it is possible that a prolonged high concentration of the contrast substance increases the risk of such injury (7)

In accordance with Langecker et al. (16) we found that hypaque injected intravenously into rabbits with normal renal function is to some small extent excreted via the liver and the bile passages. Our experiments also show that there is an excretion to the intestine besides the hepatic excretion. The amounts of contrast medium which in normal experimental animals are not eliminated through the kidneys are, however insignificant (less than 1 %) compared with those that are excreted through the kidneys. In the nephrectomized animals, on the other hand these other routes of elimination become increasingly important so that in the first 24 hours no less than a quarter of the injected dose is excreted via the liver About one-seventh of the injected dose also enters the intestine along other paths.

Our experiments further indicate that the contrast medium which thus enters the intestine can be re-absorbed (about half of the dose administered via the common duct) Langecker et al (16) showed that of orally administered 3,5-diacetylamino-2 4 6-triiodobenzoic acid in rats only 5 2 % is excreted in the urine during 22 hours. This low excretion can be explained by the fact that the substance concerned was precipitated by the acidity of the stomach (see, for instance Liebster et al. (17) for an analogous *in-vitro* experiment) and that this precipitate could not pass back into solution during its passage through the intestine. Accordingly an entero-hepatic re-circulation can in the anuric subject delay further the elimination of the contrast medium.

Because hypaque is to such a small extent excreted via the liver and the bile passages in persons with normal renal function this ^{131}I labelled contrast medium has been recommended for use in obtaining radioactive renograms (9) In cases of markedly impaired renal function however the extrarenal ways of elimination will probably — if in this respect the conditions are analogous in man and rabbits — increase in importance to such an extent that the radioactive renogram can give erroneous results. This would hold true of the right kidney in particular because of its position near the liver and biliary tract, especially if measurement is made without pulse height analysis and adequate collimation

If as mentioned earlier a prolonged high concentration of the contrast substance involves an increased risk of renal

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From the University Clinic for Internal Medicine, Wilhelmina Gasthuis, Amsterdam, Holland

Metastasizing Neuroblastoma with Excretion of 5-Hydroxyindoleacetic Acid, Serotonin and 5-Hydroxytryptophan

By

J. C. Birkenhäger

In neoplastic disease increased excretion of 5-hydroxyindoleacetic acid thus far has been connected mainly with metastasizing carcinoid tumours of the gastrointestinal tract or of bronchial origin. Endocrine activity of neuroblastomata, tumours derived from orthosympathetic ganglion cells, has revealed itself in secretion of pressor amines or their precursors. We had the opportunity to study the case of a male adult suffering from a metastasizing abdominal neuroblastoma, who had a normal urinary output of pressor-amines but a moderately increased urinary excretion of 5-hydroxyindoleacetic acid, and who furthermore excreted serotonin and 5-hydroxytryptophan.

Case report

A 56-year-old tiler who for about twenty years intermittently had complained of moderate colic-like attacks of abdominal pain with diarrhoea and who in the last two years had developed a productive cough and dyspnoea on exertion, noted in May 1962 a progressive, slightly painful distension of the abdomen. The body-temperature was often a little elevated and the patient suffered from loss of appetite, while at times he passed frequent loose stools. No typical flushing attacks were noted with certainty. Shortly before admission the urine acquired a brown, "brandy-like" colour. At the time of admission (June 26, 1962) the patient appeared moderately ill. Blood pressure was 180 mm systolic, 95 mm diastolic. Emphysema pulmonum and bronchitis were found. No heart murmurs were heard. The abdomen appeared grossly distended and painful due to an enlargement of the liver to a level surpassing the right iliac crest. Several noduli could be noted on palpation of this organ and we heard a perihepatitic rub. Since his admission the patient had no diarrhoea and no flushing attacks. Abnormal laboratory findings were: A haemoglobin level of 12.4 g %, a leucocytosis of 12,500, raised serum alkaline-phosphatase (22—50 Bessey Units), serum glutamate-pyruvate-transaminase and glutamate-oxaloacetate-transaminase (41—109 and 62—119 units respectively) and bromosulphalein retention at 45 minutes (20 %). Prothrombin activity amounted to 46 %. Total serum protein was 6.9 g % and electrophoresis at first did not

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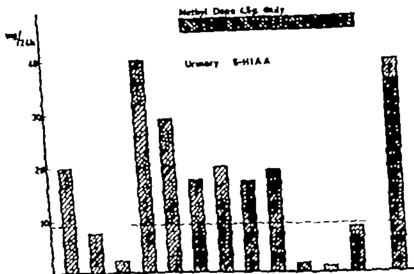


Fig. 2. Excretion of 5-hydroxyindoleacetic acid before, during and after administration of α-methyldopa.

3.7, 8.1 and 17.5 mg 5-HTP thus found, the recovery of 5-HTP added before chromatography being 75—100%. These values must be regarded as approximate. Normally less than 1.5 mg are excreted per day. The highest excretion value for 5-HTP, accompanied by a sharp decrease of that for serotonin, was found in period of administration of α-methyldopa (3 g daily) and d,l-tryptophan (2 g daily).

A daily urinary pressor-amine output equivalent to 73 and 218 μg noradrenaline was found by bioassay (modified method of Floyer (6), with pithed rats). Urinary (nor)-metanephrine (0.5 mg/24 hrs) and 3-methoxy-4-hydroxymandelic acid (VMA) (0.3 mg/24 hrs) were in the normal range.

Using the method of Udenfriend et al. (35) we detected no serotonin in the primary tumour. When deproteinized tumour extract was chromatographed in the same manner as the urinary indoles, only a tryptophan spot was visible. Assay of pressor amines by the above-mentioned method after acid extraction of the tissue resulted in an atypical response probably not due to adrenaline or noradrenaline as the response could not be suppressed by regitine. It should be mentioned that all analyses of the tumour tissue were done after the tumour had been stored frozen for at least six weeks.

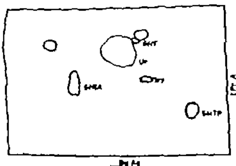


Fig. 3. Chromatogram of 0.03 ml urine (from 950 ml excreted in 24 hrs). Solvents: Isopropanol-ammonia and butanol-acetic acid; spray: Ehrlich reagent (Jepson (16)).

5-HIAA = 5-hydroxyindoleacetic acid.
5-HTA = serotonin.
5-HTP = 5-hydroxytryptophan.
IS = Indoxyl sulphate.
NI = not identified.
Try = tryptophan.
U = urea.

Discussion

In neuroblastomas and ganglioneuromas endocrine activity is well known. Since Mason et al. (18) described in an



Fig 1 Neuroblastoma tissue showing rosettes. Haematoxylin and eosin

reveal an abnormal pattern. There was no occult bleeding We did not find porphyri nuria. Radiography of the chest showed abnormal translucency of the lungs. A plain film of the abdomen and a barium meal were normal.

Repeated examination of the urine by the method of Udenfriend et al. (32) showed on most occasions the presence of a moderately increased amount of 5-hydroxyindoleacetic acid (5-HIAA) (see below)

A provisional diagnosis of atypical carcinoid syndrome was made. The condition of the patient deteriorated slowly The abdominal circumference remained the same during the period of observation, but five weeks after admission the patient developed oedema of the legs, which appeared to be due to hypalbuminaemia, arising in the course of a few weeks, and possibly also to compression of the vena cava inferior Treatment with diuretics and infusions of human albumin were without effect. In mid August the patient grew confused and needed increasing doses of analgesics for abdominal pain, and on September 7 he died after the rapid development of a uraemic state.

At necropsy there were noted wasting of the body distension of the abdomen and oedema of the legs. The lungs were oedematous. The heart was not enlarged and the valves and the endocardium did not show any abnormality About 2 litres of haemorrhagic ascitic fluid were found. The liver weighed 7 400 g and showed numerous rounded metastatic growths 1 to 10 cm in diameter A yellow-white retroperitoneal tumour mass, up to about 10 cm in diameter had infiltrated the spleen (180 g) the tail of the pancreas and the stomach wall. In the stomach wall at the place of infiltration by the tumour an ulceration was found. Otherwise the whole of the digestive tract appeared normal. Elsewhere, no tumour deposits were found. The adrenals were normal in appearance and size. In the cortex of the left kidney a small nodule was found which histologically appeared to be an adenoma. The first mentioned infiltrating tumour as well as the hepatic metastases consisted of cells with round or ovoid dark nuclei and a small zone of cytoplasm. A considerable part of the tissue was necrotic. Masses of tumour cells were separated by narrow connective tissue septa. In several places rosettes of nuclei were seen (fig 1) With the Gomori technique some fibres faintly appeared to be present in the centre of these rosettes. No argentaffin granules could be demonstrated by the Fontana method. The tissue structure was that of a neuroblastoma.

The daily excretion of 5-HIAA (32) varied between 2.3 and 40 mg (normal values 2—9 mg) Because of the spontaneous fluctuations of the excretion it was difficult to see whether administration of α-methyldopa, (Merck, Sharp & Dohme, Holland), known to inhibit the decarboxylation of 5-hydroxytryptophan (5-HTP) to serotonin (20) resulted in diminishing urinary 5-HIAA. Without treatment 23 ± 15.9 mg (mean ± S. D) were excreted, during treatment with α-methyldopa 12 ± 8.2 mg (fig 2)

Bidimensional indole chromatography of the urine by the method of Jepson (16) revealed the presence besides 5-HIAA, of serotonin (5-HTA) and 5-hydroxytryptophan (5-HTP) (fig 3) The serotonin and 5-HTP spots were not visible in all specimens and especially the intensity of the 5-HTP spot varied considerably For the daily serotonin excretion, determined according to Udenfriend et al. (33) we found on three occasions 3.2 2 2 and less than 0.3 mg (normally below 0.1 mg) We estimated the excretion of 5-HTP in the above-mentioned chromatography of the urine by el tion of the 5-HTP spots of several chromatograms together and measurement of the colour reaction with α-nitrosonaphthol (33) On the same occasions as mentioned for serotonin, daily excretions of

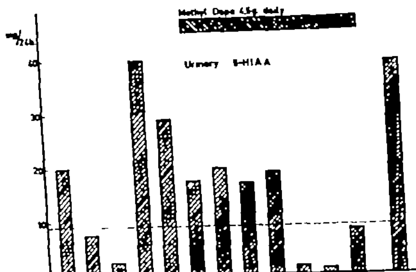


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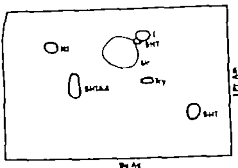


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infant paroxysmal hypertension due to the release of pressor-amines by a mediastinal tumour which appeared to be not a phaeochromocytoma but a neuroblastoma many neuroblastomas or ganglioneuroblastomas secreting pressor amines have been observed in children (8 12 15 29 30 34 35) In 1957 Wilkins (39) mentioned a case of an adrenal neuroblastoma with a positive benzodioxane test, but with only a minute amount of pressor-amine in the tumour Other workers proved the tumour to be the source of the increased secretion of noradrenaline and adrenaline (10 12 18) In several children with neuroblastoma with or without an abnormal excretion of pressor-amines and metabolites thereof an increased excretion of the precursor dopamine (3-hydroxytyramine) and its metabolites was also found (1 30 31 36) There remained cases of neuroblastoma however without an increased excretion of pressor amines VMA, (nor) metanephrine or dopamine (35 36) In our case, as mentioned no increased urinary excretion of pressor amines, VMA and (nor)metanephrine was found We did not investigate urinary dopamine or its metabolites. In the studies of Voorhess and Gardner (37) dopamine excretion in neuroblastoma paralleled that of noradrenaline.

Meanwhile attention had also been called to chronic diarrhoea as the presenting symptom in several patients (all children) with neuroblastoma, ganglioneuroblastoma or ganglioneuroma (11) Greenberg and Gardner (12 13) Gardner et al (8) and Stickler et al. (29) showed the diarrhoea to be associated with an increased excretion of catechol amines whereas these authors did not detect a pathological urinary excretion of 5-HIAA or the presence of serotonin in the tumour In general the diarrhoea did not appear to be caused by a neoplastic invasion of the coeliac and mesenteric plexuses as several of these tumours had a mediastinal localization without involvement of the posterior abdominal wall In our patient increased bowel movements had been present in the past, but at the time of rapid meta static tumour growth there was only occasionally a mild diarrhoea.

The impairment of the functional state of the liver by the extensive and numerous metastases of the neuroblastoma cannot be held responsible for the alterations in 5-hydroxyindole metabolism observed in our case, as it has repeatedly been shown that for instance in hepatic cirrhosis the excretion of endogenous 5-HIAA falls within the normal range (2, 4 27) As far as we know no abnormal endogenous excretion of serotonin or 5-HTP has been described in liver insufficiency

A remarkable feature, in view of the pathological urinary excretion of 5-HIAA serotonin and 5-HTP is the absence of flushes in our patient during the stay in hospital. After Smith et al. (28) found 5-HTP in the urine of a patient with a carcinoid syndrome and an unknown site of the original tumour Sandler and Snow (26) and Oates and Sjoerdsma (21) described cases of gastric carcinoid without diarrhoea, with a typical flushes and an abnormal excretion of 5-HIAA, serotonin and the serotonin precursor 5-HTP A similar atypical carcinoid syndrome, reported by Waldenström et al in 1956 (38) later also turned out to be caused by a metastasizing gastric carcinoid while afterwards a raised urinary output of 5-HTP was demonstrated Sandler and Snow (26) as well as Oates and Sjoerdsma (21) postulated the secretion of 5-HTP by

this type of carcinoid tumour with subsequent decarboxylation of this substance to serotonin elsewhere in the body. This was mainly on the grounds of the resemblance which the excretory pattern of 5-hydroxyindoles, after infusion of 5-HTP in control persons, bore to the pattern encountered in the above mentioned patients, viz. a large amount of 5-HTP and serotonin and a moderately increased amount of 5-HIAA as opposed to a large amount of 5-HIAA only in the typical carcinoid syndrome with serotonin-secreting tumour tissue. Direct evidence of 5-HTP output by tumour tissue was for the first time given by Sandler et al. (25) who demonstrated the presence of 5-HTP in low concentration in a hepatic metastasis of a bronchial carcinoid tumour. This patient had excreted all three 5-hydroxyindoles in large amounts. This was followed by the detection of serotonin and 5-HTP in a metastasizing pancreatic duct neoplasm which had caused a typical carcinoid syndrome also with secretion of all three 5-hydroxyindoles (23). There are, furthermore, several reports of carcinoid syndrome associated with pancreatic, bronchial and other carcinomata, all with extensive metastases to the liver (3, 14, 19, 24). The excretion of 5-HTP in our patient indicates secretion of 5-HTP by the neuroblastoma. The absence of typical as well as atypical flushes possibly is explained by the relatively low excretion of total 5-hydroxyindoles as compared with that generally seen in the carcinoid syndrome.

With regard to the absence of argentaffin granules in our case of neuroblastoma, we would stress the fact that owing perhaps to the rapid disappearance of the reaction after death, even in proven cases of carcinoid tumour this staining may be negative. Also there is no strict correlation between the absence of argentaffin granules and the secretion of 5-HTP instead of serotonin by the tumour (25), although none of the above-cited 5-HTP-secreting carcinoid tumours showed the argentaffin reaction.

Neuroblastomas or sympathicoblastomas are derived from the sympathoblast, the precursor cell of the fully differentiated sympathetic ganglion cell. It is reasonable to suppose that if the abnormal production of 5-HTP and serotonin in our patient indeed can be ascribed to the neuroblastoma tissue normal sympathetic ganglion cells also may have the capacity to synthesize 5-HTP and serotonin. An indication for this was already given by the results of the perfusion studies of Gertner et al. (9) who, with the use of iproniazid to prevent serotonin breakdown, demonstrated the production of serotonin in the superior cervical ganglion of the cat. On the other hand no serotonin was found by Gaddum and Paasonen (7) on direct analysis (with bioassay) of mammalian sympathetic ganglia. Serotonin has been implicated in the function of synapses in the brain following the well-known studies of the distribution, the release and the antagonists of serotonin in the central nervous system by the groups of Page, Gaddum, Brodie and Sjoerdsma (cf. 22). Probably serotonin has an inhibitory function in certain cerebral synapses (17) but in sympathetic ganglia its function is still uncertain (5).

Summary

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Summary

A 56-year-old male patient with an abdominal neuroblastoma and metastasis to the liver is described. Although no typical flushes nor diarrhoea were noted, 5-hydroxytryptophan, 5-hydroxytryp-

tamine (serotonin) and an abnormal amount of 5-hydroxyindoleacetic acid were found in the urine. The excretion of pressor amines, 3-methoxy-4-hydroxy mandelic acid and (nor)metanephrine appeared to be essentially normal. In the tumour tissue no 5-hydroxytryptophan or serotonin was detected. The implications of the excretion of the 5-hydroxyindoles in this case are discussed

Acknowledgements

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Evaluation of the Effect of Heparin in Addition to Oral Anticoagulant Therapy in Acute Myocardial Infarction

By

Sverre Nordöy and Arne M. Bentstad

Anticoagulant treatment in acute myocardial infarction is carried out with oral anticoagulants (dicoumarol phenylindanedione, etc.) with combination of oral anticoagulants and heparin or with heparin alone. There still exists a difference of opinion as to which method is preferable (1 2, 3 4 6 7). From a practical and economic point of view the administration of heparin represents a considerable burden and also for this reason we feel the need of a definite proof that justifies heparin treatment in acute myocardial infarction.

In the present investigation we have posed this question without discussing the benefit of anticoagulant treatment in general. The investigation has been carried out in the medical departments VIII and IX, Ullevål Hospital, Oslo.

Material

In the period between 1st Sept. 1960 and 31st Aug. 1961 268 patients were admitted to department VIII and 212 patients to department IX because of acute myocardial infarction. In department VIII 198 patients were treated with oral anticoagulants most received dicoumarol, the others phenylindanedione. In department IX 131 patients received oral anticoagulant treatment with phenylindanedione. In addition, the patients in department IX were treated with heparin intravenously during the first three days after the admission.

Twenty-three patients in department VIII and 18 patients in department IX were dead on admission. Eleven patients in department VIII and 27 patients in department IX died within 18 hours of admission and are excluded from the investigation. Most of these patients did not receive anticoagulant therapy at all because of the rapid deterioration of their condition. A smaller group received oral anticoagulant therapy but we do not believe that oral anticoagulants produce any effect during the first 18 hours (5). Eleven of the patients excluded received heparin before death in department IX one received three injections, the others only two or one injection. We do not believe that this treatment could possibly have influenced the course of the disease.

Twenty-nine patients in department VIII and 16 patients in department IX lived longer than 18 hours after the admission

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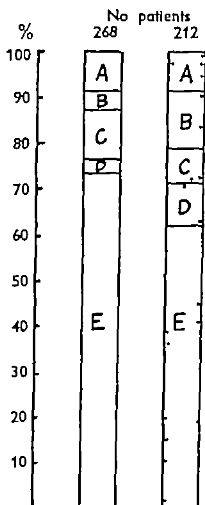


Fig 1 All patients with acute myocardial infarction admitted to med. dept VIII (left) and med. dept IX (right) from Sept. 1st 1960 t Aug 31st 1961 A = Died prior to admission. B = Died within 18 hours of admission. C Not treated with anticoagulants. D = Treated with anticoagulants in a different way from group E. E = Treated with oral anticoagulants lone (dept. VIII) or combination f oral anticoagulants and heparin (dept. IX)

but were *not* given anticoagulant treatment because of contraindications (practically all of them because of old age and/or mental reduction) A few patients who received heparin treatment in department VIII and only oral anticoagulant treatment n department IX are also excluded from the in estigation (fig 1)

Methods

Control of the anticoagulant treatment

In both departments anticoagulant therapy was started as soon as the diagnosis was verified. In department VIII the initial dose of dicoumarol was 240 mg (in a few cases 200, 220 or 260 mg) and the following doses were depending upon the PP values. In department IX the initial dose of phenylindanedione was 160 mg (in some cases 120 or 140 mg) and heparin was administered intravenously three times a day during the first three days, the daily dose being 150 + 100 + 150 mg The intensity of the oral treatment was controlled by the PP method (Owren and Aas) and in some instances by the thrombo-test method a stable level of about 15 being taken as the desirable value.

Comparability

1) The patients in both groups originate from Oslo, and nearly all of them were referred to the hospital as emergency cases. Such cases are admitted to the two departments at random

2) The average age of the patients was practically the same in both groups. In males, the average age was a little higher in department VIII than in department IX. As to the females, the opposite was the case. In the patients who died, the average age in the males was a little higher in department VIII than in department IX As to the females the opposite was the case. In both departments the average age of the patients who died was higher than that for all patients (table I)

3) The age distribution was nearly the same in both groups, about two-thirds of the patients being between 51 and 70 years old (fig 2)

4) Th sex distribution was practically the same n both groups, about 75 being males and 25 females (fig 3)

5) In different age groups the relation between numbers of males and females was very similar There was a great predominance of males between the age of 31 and 60 (fig 4)

6) 45 of the patients n department VIII and 36 of the patients in department IX had previously suffered from acut myocardial nfarction and/or had cardiac failur on admission (fig 5) Among these

Table I. Total number and mean age of all patients and the patients who died during the hospitalization

	Males		Females		Total	
	Dept. VIII	Dept. IX	Dept. VIII	Dept. IX	Dept. VIII	Dept. IX
No. of pat.	150	96	48	35	198	131
Mean age (yrs)	62	61	64.5	69	62.5	63.5
Deaths No.	23	20	8	3	31	23
Deaths %	15.3	20.8	16.6	8.6	15.6	17.5
Deaths (mean age)	65.5	61	69	74	66.5	64

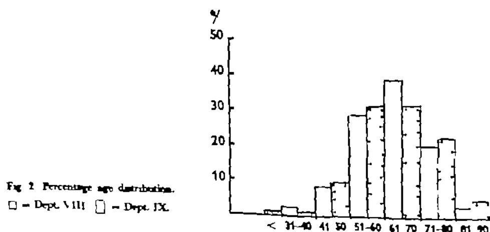


Fig. 2. Percentage age distribution.
▢ = Dept. VIII ▯ = Dept. IX.

patients the mortality rates were 25.5 and 32 respectively. Among the other patients the mortality rates were 8 and 9 % respectively (fig. 6). The percentage distributions of the patients who had previous myocardial infarction (fig. 7A) or cardiac failure on admission (fig. 7B) or both previous myocardial infarction and cardiac failure on admission (fig. 7C) were very similar in the two departments. The mortality rate within each of these groups was somewhat higher in department IX, but the differences are not significant (fig. 8).

7) Fig. 9 shows the intensity of the oral anticoagulant therapy in the patients who died. The treatment was carried out with equal intensity in the two departments, the PP-values being within the therapeutic range from the 3rd—4th day

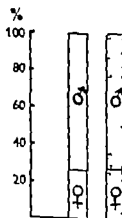


Fig. 3. Percentage sex distribution. Symbols as in fig. 2.

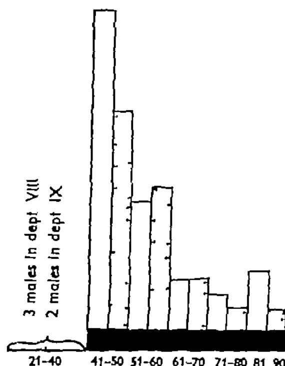


Fig 4 The ratio males females t different ages. ■ = Females (= 1) □ = Males Dept. VIII [3] = Males Dept. IX.

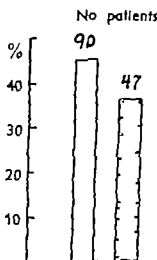


Fig 5 Percentage fraction f patients with previous myocardial infarction and/or cardiac failure on admission. Symbols as in fig 2.

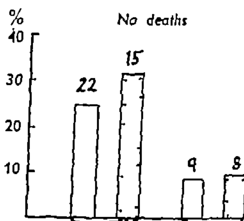


Fig 6. Comparison of the mortality rates in the two patient groups. Previous myocardial infarction and/or cardiac failure on admission (left) and no history of previous myocardial infarction or cardiac failure on admission (right) Symbols as in fig 2.

Results

Incidence of complications and mortality rate

15 6 per cent of the patients in department VIII and 17 6 per cent of the patients in department IX died during the hospitalization. As to the males, the mortality rate was somewhat higher in department IX than in department VIII As to the females the mortality rate was nearly twice as high in department VIII as in department IX (table I)

Among the patients who died post mortem examination was performed in 23 cases in department VIII and in 19 cases in department IX Recent myocardial infarction was found in all cases. Table II shows the incidence of severe coronary atherosclerosis, coronary thrombosis, intracardial mural thrombi myocardial rupture and peripheral embolism among the dead who were examined *post mortem* These findings were equally frequent in both departments The cause of death could not be related to the treatment in any case.

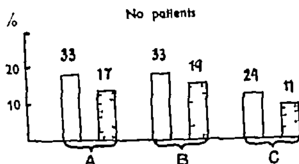


Fig 7 Percentage distribution of patients with A. Previous myocardial infarction (no heart failure); B. Heart failure on admission (no previous infarction); C. Previous myocardial infarction and heart failure on admission. Symbols as in fig 2.

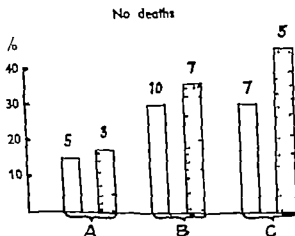


Fig 8 Mortality rates in the patients belonging to groups A, B and C in fig 7 Symbols as in fig 2

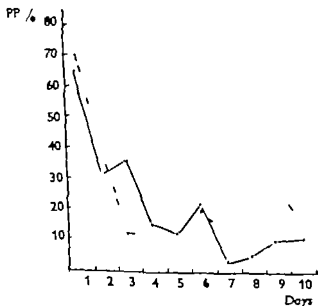


Fig 9 The intensity of the oral anticoagulant treatment in the patients who died. The relatively low average values on the first day after the admission are due to the low values in few patients on long-term anticoagulant treatment Unbroken lines dept. VIII (62 estimations) Broken lines dept. IX (91 estimations)

Table II Post-mortem examinations

	No. of deaths	No. of post mortem examin.	Severe coronary ath. scler	Coronary thrombosis	Mural thrombus	Peripheral embolism	Myocard. rupture
Dept. VIII	31	23	19	19	3	0	3
Dept. IX	23	19	18	14	2	0	2

Table III Occurrence of complications

	Survivors		Deaths		Total	
	Dept. VIII	Dept. IX	Dept. VIII	Dept. IX	Dept. VIII	Dept. IX
No. of pat.	167	108	31	23	198	131
Thrombosis/embolus	3	0	0	0	3	0
Recurrent myocard. inf. in dept.	2	6	0	2	2	8
Haemorrhage	1	3	0	0	1	3
Deaths caused by treatment	0	0	0	0	0	0

We have studied the records with regard to possible occurrences of thrombosis, embolism or haemorrhage during the period of investigation In a retrospective study there will always be the possibility of overlooking complications or evaluating them erroneously Nevertheless, we are sure that all complications of clinical importance have beeen recorded because all the patients in the material have been superintended by us during the course of the disease.

The complications are few in both groups (table III) In department VIII three cases of superficial thrombosis in the leg were recorded One of these patients had Buerger s disease. In department IX no cases of thrombosis were recorded In department IX 8 patients had recurrent myocardial infarction during the hospitalization. In department VIII only two such episodes were recorded However the small numbers do not allow any conclusions to be drawn.

In department VIII one case of haemorrhage due to a duodenal ulcer was recorded during the anticoagulant treatment. In department IX haemorrhage occurred in three patients (gross haematuria and subcutaneous haematomas) In the latter cases the haemorrhage occurred in two patients two and six days respectively after the heparin treatment was discontinued Small and quite unimportant haemorrhages, i. e. small haematomas at the site of the injections and microscopic haematuria are not included.

During the first 5 days after the admission the distribution of deaths was different in the two departments. During the rest of the stay the distribution was about equal (fig 10) On the 2nd and the 3rd day 10 patients died in department VIII while only 2 patients died

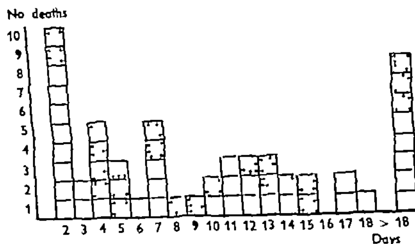


Fig. 10. Distribution of all deaths during the hospitalization. (First day after the admission excluded.) Symbols as in fig. 2

Table IV. Clinical picture and findings on post-mortem examination compared between departments VIII and IX

	No. of deaths	No. of post mortem examin.	Coronary thrombosis	Cardiogenic shock	Heart failure	Sudden death	Thrombo-embolic compl.
2nd – 3rd day							
Dept. VIII	10	7	6	6	3	1	0
Dept. IX	2	1	1	2	0	0	0
4th – 5th day							
Dept. VIII	1	1	1	myocardial rupture			0
Dept. IX	7	7	5	4	1	1	0

in department IX. On the 4th and the 5th day 7 patients died in department IX, while only one patient died in department VIII. This combination is remarkable, the possibility of its occurrence by chance being about only 0.6 per cent. Therefore, it is possible that the higher mortality rate in department VIII on the 2nd and the 3rd day is due to the omission of heparin treatment and that the higher mortality rate in department IX on the 4th and the 5th day is due to the discontinuation of heparin on the 3rd day.

To evaluate this possibility the clinical picture was compared with the post mortem findings in the patients who died during these days (table IV). In both departments, the majority of these patients

had severe cardiogenic shock and some others had heart failure with pulmonary oedema on admission. The cardiogenic shock and the heart failure proved to be progressive and irreversible, and on post mortem examination recent myocardial infarction with extensive and severe myocardial damage and massive coronary atherosclerosis was found The coronary occlusion was caused by recent thrombosis in nearly all the cases in both departments. In no cases were peripheral thromboembolic complications, especially pulmonary embolism the cause of death. Therefore, any influence of heparin treatment upon the course of the disease in these patients seems to be ruled out In other words, the unequal distribution of deaths during 2nd 5th days after the admission is possibly accidental More over the numbers are rather few and statistical calculations are therefore not warranted.

Summary and conclusion

In the present investigation oral anti coagulant treatment alone has been compared with oral anticoagulant treat ment combined with heparin given intravenously during the first three days of treatment *in acute myocardial infarc tion*.

The two patient groups are randomized and comparable with regard to average age age distribution sex distribution the male female ratio at different ages the numbers of patients with previous myocardial infarction and/or heart failure on admission and to the intensity of the oral anticoagulant treatment in the patients who died during the hospitaliza tion

No significant difference with regard to the total mortality rate or the incidence of thromboembolic and haemorrhagic complications was recorded.

The unequal distribution of deaths in the two patient groups during the first days of treatment could possibly be due to the omission of the heparin treatment in the first group and to the discontinuation of this treatment in the second group on the 3rd day However the clinical and post mortem findings contradict this possibility

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The Effects of Nicotinic Acid, Phentolamine and Nethalide on the Plasma Free Fatty Acids and the Blood Pressure in the Dog

A Comparative Study

By

Sven Fröberg and Lars Orö

During recent years it has become evident that lipids are mobilized from the adipose tissue in the form of free fatty acids (FFA) (9 13) This release of fatty acids requires lipolysis of the stored glycerides. Investigations support the concept that lipid mobilization from adipose tissue is influenced by the sympathetic nervous system (12) Epinephrine as well as norepinephrine increases the plasma FFA concentration (7 10 14 18) Catecholamines stimulate the release of fatty acids from adipose tissue in vitro (11 21) There is evidence that the increased FFA concentration in vivo caused by catecholamines is due to an enhanced lipolysis in the adipose tissue in vivo (5) It has been reported that adrenergic blocking agents can inhibit the augmented release of FFA from adipose tissue caused by catecholamines (13 15, 16 19 20) However all these drugs also have an inhibitory effect on the blood pressure changes caused by catecholamines

It was recently demonstrated that administration of nicotinic acid to man, in acute experiments, decreased the plasma FFA concentration (4) It was also found that nicotinic acid injected intravenously in dogs inhibited the FFA concentration rise caused by norepinephrine infusion without effect on the blood pressure rise. These results suggested that nicotinic acid inhibited the effects of norepinephrine on the FFA release from adipose tissue This was proven to be the case by in vitro experiments with adipose tissue (6)

In 1948 Ahlquist suggested that the effects of different adrenergic stimulating and blocking agents could be explained as an action on alpha and/or beta adrenotropic receptors (1) According to this terminology norepinephrine stimulates almost only the alpha adrenotropic receptors. Phentolamine, an alpha receptor blocking agent, inhibits the effect of norepinephrine on the blood pressure. Epinephrine stimulates alpha as well as beta adrenotropic receptors. The blood pressure rise caused by epinephrine is inhibited by phentolamine and epinephrine then

had severe cardiogenic shock and some others had heart failure with pulmonary oedema on admission. The cardiogenic shock and the heart failure proved to be progressive and irreversible, and on post mortem examination recent myocardial infarction with extensive and severe myocardial damage and massive coronary atherosclerosis was found The coronary occlusion was caused by recent thrombosis in nearly all the cases in both departments. In no cases were peripheral thromboembolic complications, especially pulmonary embolism, the cause of death. Therefore, any influence of heparin treatment upon the course of the disease in these patients seems to be ruled out. In other words, the unequal distribution of deaths during 2nd 5th days after the admission is possibly accidental. Moreover the numbers are rather few and statistical calculations are therefore not warranted

Summary and conclusion

In the present investigation oral anticoagulant treatment alone has been compared with oral anticoagulant treatment combined with heparin given intravenously during the first three days of treatment in acute myocardial infarction

The two patient groups are randomized and comparable with regard to average age, age distribution sex distribution the male female ratio at different ages, the numbers of patients with previous myocardial infarction and/or heart failure on admission and to the intensity of the oral anticoagulant treatment in the patients who died during the hospitalization.

No significant difference with regard to the total mortality rate or the incidence of thromboembolic and haemorrhagic complications was recorded

The unequal distribution of deaths in the two patient groups during the first days of treatment could possibly be due to the omission of the heparin treatment in the first group and to the discontinuation of this treatment in the second group on the 3rd day However the clinical and post mortem findings contradict this possibility

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After one hour nicotinic acid was injected intravenously. In a second series of experiments there were two periods of catecholamine infusion, each of twenty minute duration and two hours apart. The catecholamines norepinephrine and epinephrine were given at the same rate and in the same dose as in the previous series. One hour before the second infusion period the administration of the different drugs (nicotinic acid, phentolamine and nethalide) was started. The total dose was divided into twelve portions and each injected at five minute intervals.

Blood samples for analyses were withdrawn from the arterial catheter into heparinized syringes and then centrifuged immediately. The total blood volume taken from the dog was about 100 ml in each experiment.

In the third series of experiments the effects of nicotinic acid, phentolamine and nethalide on blood pressure changes caused by single injections of catecholamines were studied. In these experiments no blood samples were taken.

Substances Nicotinic acid was used as the sodium salt at pH 7 in concentration of from 5 to 20 g/100 ml. Phentolamine was kindly supplied by Ciba Stockholm as Regitin®. Nethalide (2-isopropylamino-1-(2-naphthyl) ethanol HCl) new beta adrenotropic receptor blocking agent (2, 8) was kindly supplied by Meda Goteborg Sweden as substance I.C.I 38174 (= Alderlin).

Analytical The plasma free fatty acids were determined according to Dole (7). Addition in vitro of the investigated substances did not influence the titration values.

Results

Effect of nicotinic acid on the plasma FFA concentration and the blood pressure during norepinephrine infusion

Two doses of nicotinic acid, 1 and 16 mg/kg body weight, were given during constant infusion of norepinephrine (fig 1). Nicotinic acid caused a rapid decrease of the elevated FFA concentration. The decrease lasted longer with the higher dose. No change was observed on the blood pressure.

Table I. Effect of phentolamine on changes of arterial plasma FFA concentration (mEq/l) caused by norepinephrine in the dog. Norepinephrine was infused at a rate of 0.6 µg/kg/min. during two twenty minute periods. Two dogs received 4 mg/kg and two 16 mg/kg phentolamine between the infusion periods. The individual changes are seen in the table

Dog no.	Dose phentolamine (mg/kg)	First norepinephrine infusion: Concentration before	First norepinephrine infusion: Maximum concentration	Second norepinephrine infusion: Concentration before	Second norepinephrine infusion: Maximum concentration
1	4	0.58	2.41	0.59	2.24
2	4	0.63	2.48	0.92	2.18
3	16	0.62	2.22	0.88	2.07
4	16	0.43	2.15	0.76	1.78

Effect of nicotinic acid on the epinephrine induced changes of the plasma FFA concentration and the blood pressure

Fig 2 shows that administration of nicotinic acid considerably reduced the increase of the plasma FFA concentration caused by epinephrine. The blood pressure rise was unaffected by nicotinic acid.

Effect of phentolamine on the norepinephrine induced changes of the plasma FFA concentration and the blood pressure

Two dogs were given 4 mg and two other dogs 16 mg/kg body weight of phentolamine between two periods of norepinephrine infusion (fig 3). The two doses caused a blood pressure fall and completely inhibited the effect of norepinephrine on the blood pressure. From table I it is evident that after administration of phentolamine there is still a marked rise of the plasma FFA concentration during the norepinephrine in-

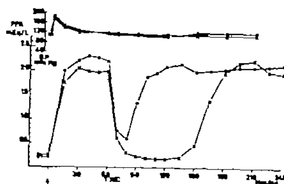


Fig 1 Effect of nicotinic acid on the arterial concentration of plasma FFA (mean value) and mean blood pressure (BP) in two dogs during norepinephrine infusion. N repinephrine (N) was infused during four hours at a rate of 0 6 μg/kg/min Two dogs received 1 mg/kg of nicotini acid (NIC) intravenously (o-o-o) The experiments were repeated one week later the dogs then received 16 mg/kg of nicotini acid (x x x)



Fig 3 Effect f phentolamine on the norepinephrine induced changes of arterial plasma FFA concentration and mean blood pressure (BP) in the dog Norepinephrine (N) was infused during two twenty minut periods at a rate of 0 6 μg/kg/min. Two dogs received 4 mg/kg and two 16 mg/kg of phentolamine before the second infusion period. Mean alue from all the four dogs was plotted (x x x.) Three dogs (controls) received only saline (o-o-o)

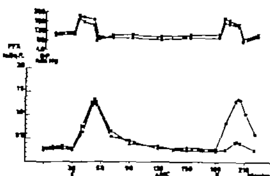


Fig 2 Effect of nicotinic cid on epinephrine induced changes of arterial plasma FFA concentration (mean value) and mean blood pressure (BP) in the dog Epinephrine (E) was infused during tw twenty minute periods at rate f 0 6 μg/kg/min Four dogs received 100 mg f nicotinic acid (NIC) intra enously as described in the text, before the second infusion period (x x) Four ther dogs (controls) received only saline (o-o-o)

causes a blood pressure fall. This so called epinephrine reversal can be inhibited by a beta blocking agent, e.g DCI (dichloroisoproterenol) or nethalide (2 isopropyl amino-1(2 naphthyl)ethanol HCl) The purpose of this investigation was to study further the pharmacological effects of nicotinic acid and to find out if nicotinic acid had any so called alpha or beta adrenotropic receptor blocking activity The effects of nicotinic acid on the catecholamine induced changes of the plasma FFA concentration and the blood pressure have been studied and compared with the effects of one typical alpha and one typical beta adrenotropic receptor blocking agent, phentolamine and nethalide respectively

Methods

Experimental. Dogs weighing between 15—25 kg were used. The morning after they had fasted overnight they were anesthetized with Nembutal® (Abbott) 30 mg/kg body weight intra enously The blood pressure was measured continuously with an Elema Schönander pressure transducer (EMT 490 A) from a tephlon catheter inserted into the femoral or brachial artery The blood pressure transducer as filled with saline and no heparin was injected into the animal.

In one series of experiments norepinephrine (1 base) was infused continuously at a constant rate 0 6 μg/kg/min., during four hours.

Effect of nicotinic acid on nethalide induced changes of the plasma FFA concentration

There was a rapid increase of the FFA concentration when 4 mg/kg of nethalide was administered (fig 6) Nicotinic acid administration completely inhibited the nethalide induced rise of the FFA concentration.

Effect of nicotinic acid, phentolamine and nethalide on the blood pressure changes caused by single injections of norepinephrine and epinephrine

Fig 7 shows that nicotinic acid 200 mg/kg body weight, did not change the blood pressure rise caused by a single injection of norepinephrine or epinephrine. As can be seen in fig 8, phentolamine, 2 mg/kg body weight, lowered the blood pressure and completely inhibited the blood pressure rise caused by norepinephrine. The same dose of phentolamine also caused so-called epinephrine reversal. It was not possibl t inhibit this blood pressure fall caused by epinephrine even with 200 mg/kg body weight of nicotinic acid However 1 mg/kg of nethalide produced complete inhibition. In other experiments it was found that the minimum dose of nethalide for this effect was from 1/10 to 1/4 mg/kg body weight.

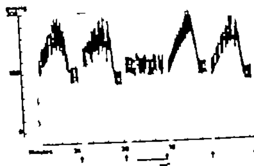


Fig. 7 Effect of nicotinic acid on changes of the arterial blood pressure caused by single injections of norepinephrine and epinephrine in the dog Time scale, from right to lef is interrupted and marked in minutes. The figures giv the time from the beginning of the experiment. At E 0.8 µg/kg epinephrine was injected intravenously N 0.8 µg/kg norepinephrine. At NIC 200 mg/kg nicotinic acid was injected.



Fig 8. Effect of nicotinic acid, phentolamine and nethalide on the arterial blood pressure changes caused by single injections of norepinephrine in the dog Time scale from righ to left is interrupted and marked in minutes The figures represen the time from the beginning of the experimen The different substances were injected intravenously as follows at N 0.8 µg/kg of norepinephrine at E 0.8 µg/kg of epinephrine, at R 2 mg/kg of phentolamine, NIC 200 mg/kg of nicotinic acid and I 1 mg/kg of nethalide

Discussion

In the present investigation the previous finding that nicotinic acid in dogs significantly inhibited the rise of the FFA concentration caused by norepinephrine (4) was confirmed.

It was also demonstrated that nicotinic acid inhibited the rise of the FFA concentration caused by epinephrine.

While 1 mg/kg body weight of nicotinic acid inhibited the norepinephrine induced FFA concentration rise, doses up to 200 mg/kg body weight had no effect on the blood pressure rise caused by norepinephrine or epinephrine. The

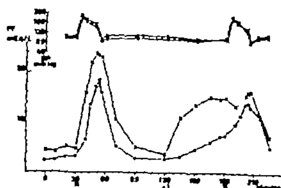


Fig 4 Effect of nethalide on the norepinephrine induced changes of arterial plasma FFA concentration and mean blood pressure (BP) in the dog Norepinephrine (N) was infused at a rate of 0 6 µg/kg/min. during two twenty minute periods. Nethalide (I) was given during the hour before the second infusion period. Two dogs received 1 mg/kg (o-o-o) and two 4 mg/kg (x x) of nethalide

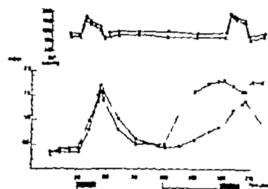


Fig 5 Effect of nethalide on the epinephrine induced changes of arterial plasma FFA concentration and mean blood pressure (BP) in the dog Epinephrine (E) was infused at a rate of 0 6 µg/kg/min during two twenty minute periods. Nethalide (I) was given during the hour before the second infusion period. Two dogs received 1 mg/kg (o-o-o) and two 4 mg/kg (x x x) of nethalide.

Effect of nethalide on changes of the plasma FFA concentration and the blood pressure caused by norepinephrine

Fig 4 shows the effect of two different doses of nethalide, 1 and 4 mg/kg body weight on the plasma FFA concentration. The two doses caused an increase of the FFA concentration. The higher dose gave a more rapid and pronounced increase

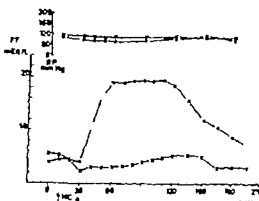


Fig 6. Effect of nicotinic acid on the nethalide induced changes of arterial plasma FFA concentration and mean blood pressure (BP) in the dog Nethalide (I) 4 mg/kg was given during one hour to three dogs (o-o-o) The total dose was divided into twelve portions and each injected at five minute intervals. One week later the same experiments were repeated except that twenty minutes before the administration of nethalide nicotinic acid (NIC) 200 mg/kg was injected intravenously (x)

of FFA. During the second norepinephrine infusion period there was a smaller rise of the mean FFA concentration than during the first period. The maximum concentration level was also lower during the second period Smaller doses of nethalide did not inhibit the rise of the FFA concentration induced by norepinephrine

Effect of nethalide on changes of the plasma FFA concentration and the blood pressure caused by epinephrine

Two doses of nethalide were given 1 and 4 mg/kg body weight It is evident (fig 5) that during the second epinephrine infusion period there was a smaller rise of the mean FFA concentration than during the first period With the higher dose of nethalide there was actually a fall of FFA during the second period The blood pressure rise was potentiated by nethalide.

Effect f nicotinic acid on nethalide induced changes of the plasma FFA concentration

There was a rapid increase of the FFA concentration when 4 mg/kg of nethalide was administered (fig 6) Nicotinic acid administration completely inhibited the nethalide induced rise of the FFA concentration.

Effect f nicotinic acid, phentolamine and nethalide on the blood pressure changes caused by single injections of norepinephrine and epinephrine

Fig 7 shows that nicotinic acid 200 mg/kg body weight, did not change the blood pressure rise caused by a single injection of norepinephrine or epinephrine. As can be seen in fig 8, phentolamine, 2 mg/kg body weight lowered the blood pressure and completely inhibited the blood pressure rise caused by nor epinephrine. The same dose of phentol amine also caused a so-called epinephrine reversal. It was not possible to inhibit this blood pressure fall caused by epinephrine even with 200 mg/kg body weight of nicotinic cid However 1 mg/kg of nethalide produced complete inhibition. In other experiments t was found that the minimum dose of nethalide for this effect was from 1/10 to 1/4 mg/kg body weight.

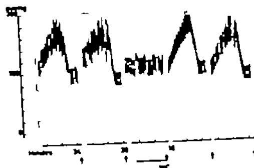


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Discussion

In the present investigation the previous finding that nicotinic cid in dogs significantly nhibited the rise of the FFA concentration caused by norepinephrine (4) was confirmed.

It was also demonstrated that nicotinic acid inhibited the rise of the FFA concentration caused by epinephrine.

While 1 mg/kg body weight of nicotinic acid inhibited the norepinephrine induced FFA concentration rise, doses up to 200 mg/kg body weight had no effect on the blood pressure rise caused by norepinephrine or epinephrine. The

blood pressure fall caused by epinephrine after previous administration of phentolamine was also unchanged. Thus according to the Ahlquist terminology (1) nicotinic acid had no demonstrable alpha or beta adrenotropic receptor blocking activity on the blood pressure.

The effects of nicotinic acid on the FFA concentration and the blood pressure were compared with the effects of one alpha and one beta adrenotropic receptor blocking agent phentolamine and nethalide. Phentolamine was administered in a dose about 20 times that necessary to inhibit completely the effect of norepinephrine on the blood pressure. Due to circulatory changes it is difficult to get any estimate of the flux of FFA through plasma from the concentration of the plasma FFA. Changes in blood flow and in distribution of blood have been discussed as an important factor for the flux of FFA (3). However it can be stated that infusion of norepinephrine without causing any blood pressure change rapidly increased the plasma FFA concentration in all the phentolamine-treated animals.

This suggests that phentolamine in contrast to nicotinic acid was not effective in blocking the effect of norepinephrine on adipose tissue in vivo.

The effect of nethalide on the plasma FFA concentration was also different from the effect of nicotinic acid. As has previously been reported with DCI (15, 20) there was a significant immediate rise of the FFA concentration when nethalide was injected. After the administration of nethalide the rise as well as the maximum of the mean FFA concentration during the second catecholamine infusion period was lower than during the first period. The results seem to indicate that nethalide was effective in blocking the effects of catecholamines on adipose tissue in vivo in the dog. It has also been reported recently that nethalide inhibited the effect of epinephrine on the plasma FFA concentration in man (17). However the difference between nicotinic acid and nethalide is obvious: first, from the fact that nicotinic acid in itself did not increase the plasma FFA concentration; secondly, that nicotinic acid inhibited the nethalide induced FFA concentration rise.

It is thus evident that the effects of nicotinic acid on the catecholamine induced changes of the blood pressure as well as the FFA concentration are different from the effects of a typical alpha and a typical beta adrenotropic receptor blocking agent. The results also demonstrate that nicotinic acid inhibits the effect of catecholamines on the FFA metabolism more specifically than hitherto investigated adrenergic blocking agents.

Summary

The effects of nicotinic acid on the catecholamine induced changes of the plasma FFA concentration and the blood pressure have been studied in the dog. Nicotinic acid inhibited the rise of the FFA concentration caused by norepinephrine as well as epinephrine. Nicotinic acid had not effect on the blood pressure rise caused by norepinephrine and epinephrine. The blood pressure fall caused by epinephrine after previous administration of phentolamine was also unchanged by nicotinic acid.

The effects of nicotinic acid have been compared with the effects of phentolamine and nethalide. The results indicated that phentolamine could not inhibit effectively the effects of norepinephrine on the FFA concentration. Nethalide

seemed to inhibit the effect of norepinephrine as well as epinephrine on the FFA concentration. However nethalide by itself caused an increased FFA concentration. This rise could be inhibited by previous administration of nicotinic acid.

Acknowledgement

The authors express their thanks to Lars A. Carlson, M. D. for stimulation and criticism of this investigation.

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Gastric Sediment Fluorescence after Administration of Tetracycline

By

ARTHUR HAHN-PEDERSEN, VAGN DREWES and EGON JENSEN

The purpose of this report is to examine the published results of experiments with tetracycline fluorescence in gastric sediment from patients with gastric carcinoma.

Klinger and Katz (2) have examined 18 patients with gastric carcinoma, 17 of whom gave a positive fluorescence reaction. A control group comprising 41 patients gave negative reactions.

In the study of 103 patients Berk and Kantor (1) found that 18 of 20 patients with cancer and 2 patients with suspected malignancy gave a positive test. Of the 81 patients with benign gastric disorders only 3 gave a positive test.

As the diagnosis of gastric carcinoma is still very difficult — certainty being in some cases achieved only by operation in spite of different methods of examination — the tetracycline fluorescence test, which is very simple, would be a valuable diagnostic help if the previously reported results were reproducible.

Submitted for publication May 3, 1963.

Material and method

Our material consisted of 6 patients with gastric carcinoma, and 18 with benign peptic ulcers (6 gastric and 12 duodenal ulcers).

The diagnosis of gastric carcinoma and gastric ulcer was confirmed at laparotomy.

The patients received 250 mg Acromycin® 4 times a day for 5 days. On the 7th day after 12 hours' fasting period, gastric lavage was carried out by the authors personally using the technique described by Rankin et al. (3). Approximately 20 ml of the mixed aspirate were centrifuged, and the sediment was transferred to a filter paper, dried and examined for fluorescence in ultraviolet light.

Results

The fluorescence reaction was positive in only 2 of the 6 cases of gastric carcinoma. Both cases showed adenocarcinoma in the antrum, the size of a hen's egg.

The 4 negative reactions were found in patients with

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The Subcutaneous Absorption of Albumin in Edematous States

By

Hans Lassgård

The purpose of this investigation has been to study the rate of the subcutaneous absorption in edematous conditions, with particular regard to the absorption of drugs with a high molecular weight.

The *subcutaneous injection of drugs* was first described by Wood in 1855 (16). Since then a great number of papers dealing with questions relating to subcutaneous absorption, especially to the absorption rate, have been published. The most extensive review has been given by Schou (13).

It is noteworthy that practically all of these authors have examined the absorption of compounds with low molecular weights. In spite of the fact that common drugs as for instance insulin and vasopressin are proteins, very few experimental studies (1, 3, 6, 9) have been carried out to elucidate the absorption of compounds with high molecular weights.

The special question of the absorption from edematous tissues has likewise been the subject of many publications but in this case also it is characteristic that only the absorption of crystalloids has been studied. The removal of proteins from edematous tissues has been considered by very few investigators (11, 12, 15) and their results are often incompatible.

Hollander *et al.* (4) by a technique similar to the one used in the present study has estimated the lymphatic function in patients with and without edema.

Methods

The absorption of albumin was examined by determination of the local clearance of ^{131}I-labelled human serum albumin. In selected cases this method was supplemented with measurements of the radioactivity in plasma and urine.

One to 10 μc of a solution of albumin was administered subcutaneously in volume of 0.2—0.5 ml which was injected 2 cm from the site of puncture. The site of puncture was marked out. Notes were made of the distance to the counter etc., so that the experimental arrangement could be accurately reproduced.

The dependence of the results on the size of the injected volume, the choice of injection site, the muscular activity etc. was studied

Submitted for publication May 6, 1963.

1) a very large inoperable adenocarcinoma in the fundus with massive infiltrations in the surrounding tissue,

2) a 3×8 cm operable adenocarcinoma in the middle of the stomach

3) a 3×4 cm ulcerated adenocarcinoma

4) a superficial colloid carcinoma limited to a smaller part of the prepyloric mucosa

Definite yellow fluorescence was found in one case of the 6 benign gastric ulcers. This ulcer did not differ macroscopically or microscopically from the other 5 ulcers, all of which gave negative reactions.

The third group consisting of 12 patients with duodenal ulcers demonstrated by X ray gave definite fluorescence in 6 of the cases.

Discussion

Klinger and Katz (2) as well as Berk and Kantor (1) found that a positive reaction cannot be obtained at a low pH in the aspirate and consequently they adjust the pH to 6 0 if necessary Using a buffer solution of 1 molar sodium acetate adjusted to pH 5 5 with acetic acid for the lavage, we found that further pH corrections were unnecessary

Klinger and Katz do not mention how much of the gastric aspirate was centrifuged (possibly all of it) We have found 20 ml to be the most practicable amount.

The positive results from patients with benign ulcers in our material show that the discrepancies in technique have been of no significance, in as much as lower pH and smaller amounts would tend to give fewer positive reactions

Vassar et al. (4) demonstrated that the yellow fluorescence in tumor tissue after tetracycline administration was not confined to the tumor cells, but to macrophages and tissue debris in the tumor stroma. In support of this, fluorescence was found in macrophages and cellular debris from non-specific skin ulcers.

It is therefore reasonable to assume that cellular debris from a large benign gastric ulcer may show fluorescence.

Regurgitation of fluorescent debris from the ulcer might be an explanation to account for the 6 positive results in the group with duodenal ulcer corresponding to the fluorescence of the gastric ulcer

Another explanation, which we have not examined, may be that fluorescence may occur in normal gastric aspirates after tetracycline administration.

The conclusion of this investigation is that fluorescence examinations of gastric aspirate after tetracycline administration is not a satisfactory method for estimating the possible malignancy of a gastric ulcer

Summary

Fluorescence of gastric sediment after administration of tetracycline was found in 2 of 6 cases of gastric carcinoma and in 7 of 18 cases of benign ulcers.

We conclude that the tetracycline fluorescence test cannot be used for differentiation between benign and malignant gastric ulcers

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Table I. The biological half-time of disappearance of subcutaneously injected albumin, labelled with ^{131}I

Group		No. of pat.	$T^{1/2}$ (hrs)
1	Normals	11	29–40
2	Cardiogenic edema	7	16–26
3	Nephrogenic edema	7	11–25
4	Thrombophlebitic edema	6	15–30
5	Myxedema	3	30–82
6	Lymphedema	3	30–105
7	*Thick legs	9	44–107

*Disabling rheumatoid arthritis, 4, severe degenerative arthritis, 2, and young, otherwise healthy women, 3

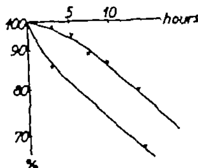


Fig. 2. Examples of the initial disappearance curve.

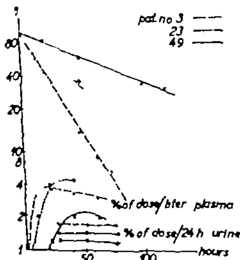


Fig. 3. Simultaneous recording of the local clearance, the plasma activity and the urinary activity after subcutaneous injection of ^{131}I-albumin in 3 patients with different rates of absorption.

the so-called "self depression" which is thought to be caused by locally released histamine which acts on the paracapillary circulation resulting in a depressed absorption rate. This initial part of the curves, however, has been omitted in the following figures.

Fig. 3 illustrates the correlation between the local clearance, the serum activity and the urine activity in three patients, one with a normal absorption rate, one with an increased and one with a decreased absorption rate. It appears that an increased absorption rate is accompanied by a high rise in the plasma activity and an early rise in the excretion, and that correspondingly a decreased absorption rate is followed by a slow build up of the plasma activity and a lower urine activity.

The biological half times of disappearance of albumin have been summarized in table I. It is seen that the six groups of patients evidently fall in two main groups, the first one consisting of the patients with heart failure, hypoalbuminemia and thrombophlebitis who all display a faster absorption rate than the normals and a second main group comprising patients with myxedema, lymphedema and those with "thick legs" who all presented with a slower rate of absorption than the normals. These relationships are also demonstrated in fig. 4 where the groups are represented by the areas for the curves.

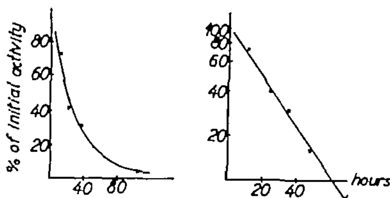


Fig 1 Example of normal disappearance curve for ^{131}I-albumin (to the right in semilogarithmic system)

in normals and the conditions chosen so that these factors could be left out of consideration.

The site of injection was externally monitored at intervals. The measured activity was corrected for "background" by subtracting the activity of the uninjected limb. The corrected activities were expressed as a percentage of the initial activity and plotted as a function of the time. A straight line was thereby obtained from which the half time of disappearance ($T_{\frac{1}{2}}$ effective) could be read directly. These values were corrected for the natural decay of the isotope by the equation $\frac{1}{T\frac{1}{2}\text{effec.}} = \frac{1}{T\frac{1}{2}\text{physic.}} + \frac{1}{T\frac{1}{2}\text{biolog.}}$ ($T\frac{1}{2}$physical = 8.08 days) whereby the biological half time ($T\frac{1}{2}$biological or simply $T\frac{1}{2}$) was determined.

In a small number of patients measurements on the rate of removal of $^{131}I^-$ were carried out in a similar way after the subcutaneous injection of Na-^{131}I.

In selected cases radioactivity of plasma and urine samples were assayed in a well type crystal and related to appropriate standards. The plasma concentration was expressed as a percentage of the injected dose per liter of plasma. The urine concentration was expressed as a percentage of the injected dose in 24 hour urine samples.

Material

The absorption rate of albumin was determined in 11 patients without edema and in 35 patients with edema of varying etiologies. Seven patients had edema secondary to congestive heart failure. Seven patients with nephrotic syndrome had peripheral edema associated with hypoalbuminemia. Six patients had unilateral edema caused by deep thrombophlebitis. Three patients had myxedema". Three females had lymphedema of an entire upper extremity following radical mastectomy for carcinoma of the breast. The remaining 9 patients presented with the well known clinical picture of "thick legs". These peculiar edema-like but non-pitting thickenings of the subcutaneous tissue of the lower extremities are seen partly in elderly immobile patients, partly in young, otherwise healthy women. Of the 9 patients belonging to this category 4 had disabling rheumatoid arthritis, 2 had severe degenerative arthritis and lastly there were 3 young women in whom no disease could be demonstrated.

Results

Fig 1 shows an example of a normal disappearance curve.

Fig 2 illustrates the fact that the initial part of the curve often deviates from the characteristic exponential curve. In some instances the initial convexity of the curve is found to be upturned, in others downturned. The explanation for this is not clear. An initially increased clearance might be because some of the radioactivity was injected directly into the blood or lymph vessels. On the other hand an initially decreased removal rate can possibly be accounted for by

precipitable with trichloroacetic acid and all of the radioactivity was contained in the albumin fraction separated by electrophoresis.

The decreased rate of absorption of albumin in lymphedema and in other conditions where a decreased lymph drainage might be suspected indicate that proteins are mainly returned to the bloodstream via the lymphatic system. This is in accordance with previous animal experimental studies where the thoracic duct lymph was collected and analyzed (1 3 6, 9) This theory is also supported by results gained by lymphangiography (8)

The increased rate of absorption of albumin in edema caused by heart-insufficiency hypoalbuminemia and thrombophlebitis is a more surprising finding. If anything one would have expected the absorption of drugs from edematous tissues to be delayed but as far as drugs with a high molecular weight are concerned the exact reverse has been demonstrated. On the ther hand the absorption of ^{131}I was found to be delayed in patients with edema due to heart or kidney diseases. This corresponds rell with previous investigations on the emoval of inorganic ions from the nterstitial fluid (10) and on the regional irculation measured by the clearance of radioactive sodium (7) The fast rate of absorption of albumin in the same conditions could be explained by suggesting an increased lymph drainage under these circumstances. This is however incompatible with the classical experiments of McMaster (11) who injected dyes directly into the lymphatics and thereby showed that in edematous conditions the drainage of lymph is reduced which in turn will aggravate the

It is probably misleading to try to explain the differences between the absorption of crystalloids and colloids from edema of different etiologies by hydrostatic and circulatory phenomena alone. The most important causal changes should perhaps be searched for in the ground substance of the connective tissue. The composition of this ground substance is altered with variations in the water contents (5)

It is noteworthy that the rate of absorption of albumin in the present study was found to be increased in the same conditions where the contents of protein in the edema fluid are decreased, but decreased in the same conditions where the contents of protein are increased (2) Calculations on a percentage basis on the absorption of the injected albumin in relation to the tissue proteins lead to the same correlations and may indicate an explanation of some of the results.

Summary

The changes n the rate of subcutaneous absorption occurring in edematous conditions have been studied.

The rat of absorption was determined by the local clearance after the subcutaneous injection of the radioactive labelled compound.

The absorption of albumin was studied in 46 patients. In 1 patients the absorption rate of Na ^{131}I was also determined.

It was shown that the rate of absorption of albumin was increased in edema caused by decompensated heart disease, the nephrotic syndrome and in deep thrombophlebitis but that the absorption was delayed in lymphedema and myx-edema. The ...

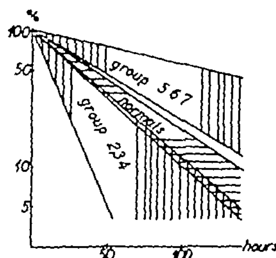


Fig 4 Graphic representation of the biological half times of disappearance in all groups.

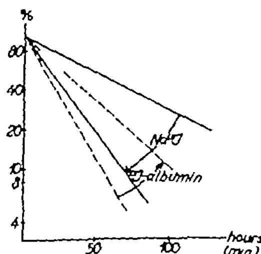


Fig 5 Disappearance curves for ^{131}I-albumin and ^{131}I$^-$ in the same patient before and after treatment for decompensated heart disease

In table II the corresponding values for the absorption of Na ^{131}I are given.

Fig 5 shows disappearance curves for ^{131}I$^-$ as well as for ^{131}I albumin from a patient with decompensated heart disease before and after treatment with digitalis During the treatment the two curves are both displaced but in opposite directions so that both of them enter the area of normal values but from opposite sides

Table II The biological half-time of disappearance of subcutaneously injected Na ^{131}I

Group		No. of pat.	T½ (min)
1	Normals	4	10, 12, 15, 18
2	Cardiogenic edema	2	37 51
3	Nephrogenic edema	2	27 37
4	Lymphedema	1	16
5	"Thick legs"	3	11 12, 14

Discussion

According to Schou (14) the rate of absorption can be studied in one of the following ways 1) recording of post absorptive symptoms 2) chemical analysis of the drug in the circulating blood 3) chemical determination of the drug in the excretions of the organism or 4) tissue clearance methods, either by local excision and quantitative chemical analysis or by recording the clearance from the surface of the injection site by using radioactive isotopes. Because a series of events such as absorption, distribution excretion chemical transformation etc. occur at the same time in the dynamic system two or more methods should be applied simultaneously

In the present study the rate of absorption has been determined by the local clearance of the radioactive labelled compound This method is theoretically the method of choice but local phenomena such as spread and diffusion are sources of error The plasma and urine activities which were determined simultaneously showed however that the blood level and excretion varied according to the local clearance (fig 3) which therefore in itself was considered to be a reliable expression of the absorption rate It was shown furthermore that 94—98 per cent of the radioactivity of the plasma was

recipitable with trichloroacetic acid and all of the radioactivity was contained in the albumin fraction separated by electrophoresis.

The decreased rate of absorption of albumin in lymphedema and in other conditions where a decreased lymph drainage might be suspected indicate that proteins are mainly returned to the bloodstream via the lymphatic system. This is in accordance with previous animal experimental studies where the thoracic duct lymph was collected and analyzed (1 3 6, 9) This theory is also supported by results gained by lymphangiography (8)

The increased rate of absorption of albumin in edema caused by heart insufficiency hypoalbuminemia and thrombophlebitis is a more surprising finding. If anything one would have expected the absorption of drugs from edematous tissues to be delayed but as far as drugs with a high molecular weight are concerned the exact reverse has been demonstrated. On the other hand the absorption of ^{131}I was found to be delayed in patients with edema due to heart or kidney diseases. This corresponds well with previous investigations on the removal of inorganic ions from the interstitial fluid (10) and on the regional circulation measured by the clearance of radioactive sodium (7) The fast rate of absorption of albumin in the same conditions could be explained by suggesting an increased lymph drainage under these circumstances. This is however incompatible with the classical experiments of M Master (11) who injected dyes directly into the lymphatics and thereby showed that in edematous conditions the drainage of lymph is reduced which in turn will aggravate the already existing edema.

It is probably misleading to try to explain the differences between the absorption of crystalloids and colloids from edema of different etiologies by hydrostatic and circulatory phenomena alone. The most important causal changes should perhaps be searched for in the ground substance of the connective tissue. The composition of this ground substance is altered with variations in the water contents (5)

It is noteworthy that the rate of absorption of albumin in the present study was found to be increased in the same conditions where the contents of protein in the edema fluid are decreased, but decreased in the same conditions where the contents of protein are increased (2) Calculations on a percentage basis on the absorption of the injected albumin in relation to the tissue proteins lead to the same correlations and may indicate an explanation of some of the results.

Summary

The changes in the rate of subcutaneous absorption occurring in edematous conditions have been studied.

The rat of absorption was determined by the local clearance after the subcutaneous injection of the radioactive labelled compound.

The absorption of albumin was studied in 46 patients. In 1 patients the absorption rate of Na ^{131}I was also determined.

It was shown that the rate of absorption of albumin was increased in edema caused by decompensated heart disease, the nephrotic syndrome and in deep thrombophlebitis but that the absorption was delayed in lymphedema and myxedema. The changes were very pronounced and must be of clinical significance

for example in administering insulin to patients with edema

It was furthermore shown that the principal route of removal of protein in man is through the lymphatic vessels and that "thick legs" and lymphedema with regard to the rate of absorption are identical.

The removal of $^{131}I^-$ was found to be delayed in edema secondary to heart and kidney diseases but these changes are smaller and probably not of clinical significance.

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Occurrence of Cryptococcus Neoformans in Sweden

By

FRANK BERGMAN

Cryptococcosis occurs in all parts of the world and Cryptococcus neoformans is the commonest cause of mycotic meningitis in human beings. Epidemiological studies have shown a fairly wide occurrence of C. neoformans in nature with a strikingly high frequency in pigeon excreta (1 5 7 13 20 24 28). This means that a fair portion of the population is exposed to the fungus and the incidence of subclinical and clinical cases of cryptococcosis is probably much higher than that suggested by the some 500 cases reported mainly from U.S.A. and Australia. A large number of these cases have occurred in association with malignant systemic diseases such as Hodgkin disease, leukaemia and lymphosarcoma (9 10 l. a.)

From Scandinavia and Finland only 10 cases with a firm diagnosis of cryptococcosis have hitherto been reported (3 4 6, 8, 11 12, 18, 21 23 27). No epidemiological investigations have been published on the spread of the fungus in these countries.

The present study was undertaken in an attempt to determine whether a reservoir of C. neoformans exists in pigeon excreta in the southernmost district of Sweden. In addition, the records and slides from an autopsy material of malignant systemic diseases have been restudied for complicating cryptococcal infections.

Submitted for publication May 8, 1963.

Material and methods

Isolation of fungi

Specimens of pigeon excreta were collected from 450 domesticated pigeons on exhibition at the Agricultural show in Malmö in Nov 1961. The pigeons, which were of 10 different breeds, had been exhibited by all together 57 owners in South Sweden (fig. 1). The birds were kept in wire cages. The bottoms of the cages were not strewn with hay or straw but with sand. The specimens, which consisted of relatively fresh droppings, were collected with sterile spatulae and stored in sterile screw-capped test-tubes of plastic in a deep-freeze refrigerator at — 25 C until processed. To each specimen were added 20 ml physiological saline and some sterile glass beads. The tubes were shaken vigorously in an agitator for 15 min. and then allowed to settle for about

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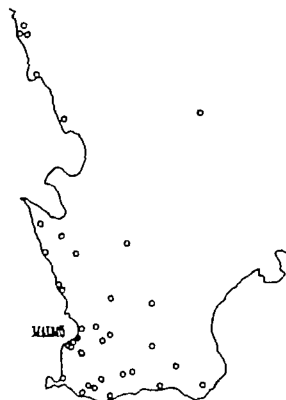


Fig 1 M p of southern Sweden Hollow circles indicat homes of pigeons solid circles, homes of pigeons whose excreta contained Cryptococcus neoformans.

15 min The supernatant fluid was streaked both heavily and lightly on two Sabouraud dextrose-agar plates containing penicillin and streptomycin to free the culture of any contaminating bacteria. The plates were sealed and incubated at 37 C and daily readings were made for four weeks. The procedure is essentially that described by Littman and Schnelerson (20)

All colonies suspected of being C. neoformans were harvested from the surface of the plates and suspended in physiological saline The solution was adjusted by dilution until 0 04 ml contained about 10,000 fungi determined microscopically in a Burker counting chamber and was then injected intracerebrally into three mice Altogether 87 mice were injected intracerebrally with solutions of 29 cultures of fungi isolated from pigeon excreta. From all animals, both from those that died and from survivors killed after three months, India ink mounts from the surface of the brain were studied microscopically after which the entire brain was fixed in 10 % formalin solution and paraffin sections were prepared for histological examination. The sections were stained with Htx-eosin, Gridley fungus stain and McManus stain.

The cultures that were virulent for mice and demonstrable in smears from the surface of the brain and in sections of the brain from animals that died were subjected to further identification tests. The fungi were transferred to Cryptococcus capsule agar slants (19) and stored at 37° C. The cellular morphology was studied and the fungi were tested for their ability to assimilate carbohydrate and nitrogen.

Those strains that could thus be identified as probably C. neoformans were sent to Centraalbureau voor Schimmelcultures, Yeast division, Delft, Netherlands, for control examination.

Check-examination of autopsy material

The material consisted of 185 consecutive cases of leukaemia, lymphosarcoma, reticulum cell sarcoma, Hodgkin s disease and multiple myeloma, autopsied during the years 1957—60 at the University Department of Pathology Malmö (table I) Sections of the lungs, liver spleen and mediastinal lymph nodes stained with Htx-eosin were re-studied, and new sections stained with Gridley' fungus stain and McManus stain were studied for C. neoformans.

Results

Isolation of fungi

From the 450 specimens collected, 29 cultures tentatively considered to be Cryptococcus neoformans were actually studied. Of these 29 13 were pathogenic for mice but only 3 of the cultures (Nos. 476 478 and 535) possessed the characteristics of C neoformans. On Sabouraud-dextrose agar plates fungi colonies were observed after 3—5 days as irregular creamy colonies with a smooth surface. The growth was not inhibited by incubation at 37°C. India ink mounts of the three cultures

revealed round to short-oval budding fungi without mycelium. The fungi showed distinct cell walls and thin capsules. The capsular substance, however increased considerably in vitro during incubation on Cryptococcus capsule medium at 37° C (19) and in vivo in mouse brain (on intracerebral injection into mice) Intracerebral injection into mice caused occipital bulging and death in most cases within 12 days, in all cases within 45 days. At autopsy encapsulated cryptococci could be demonstrated both in India ink mounts from the surface of the brain and in sections of the brain. The fungi assimilated dextrose, galactose and sucrose but failed to assimilate lactose. Nor did they reduce nitrates to nitrites.

On control examination of the fungus cultures at the Yeast Division, Centraalbureau voor Schimmelcultures, (Delft, Netherlands) it was found that they "answered completely to the description of C. neoformans given in the review by N J W Kreger-van Rij (15) Culture No. 476 showed the smallest cells in malt extract $(3.5—5.6) \times (3.8—5.7)\mu$ those of 535 and 478 were about the same size respectively $(3—6) \times (3—6.2)\mu$ and $(3.2 - 5.5) \times (4—6)\mu$. All three assimilated creatinine promptly in the auxanographic assimilation test, without showing any growth in creatine.

The culture 4 6 showed on the plate, creamy white as well as yellow colonies both belonging to Cryptococcus neoformans. The cultures 535 and 478 showed one type of colony only

The four isolated cultures of C. neoformans thus appear to represent at least two different strains of C. neoformans. It was of interest to note that all the three pigeons from whose excreta fungi had been cultured belonged to the same breeder They represented two different breeds of pigeons which were otherwise kept together with about 50 other pigeons in the same dovecot.

Table I Re-study of 185 consecutive autopsy cases

Condition	No.
Leukaemia lymphatic	18
Leukaemia myeloid	71
Lymphosarcoma	6
Reticulum cell sarcoma	37
Hodgkin disease	23
Multiple myeloma	30
Total	185

Check-examination of autopsy material

The re-study of 185 consecutive autopsy cases of leukaemia, lymphosarcoma, reticulum cell sarcoma, Hodgkin's disease and multiple myeloma (table I) revealed no evidence of cryptococcosis. In three (one case of multiple myeloma and two cases of myeloic leukaemia) Candida albicans had been demonstrated in the lungs.

Discussion

The source of human cryptococcal infection is by no means properly understood It is, however known that soil is one of the natural habitats of fungi pathogenic for human beings. The occurrence of C neoformans in pigeon excreta was regarded as an adventitious saprophyte occurring in a suitable culture medium (5) Stalb (25) showed that bird excreta or urine (canary and pigeon) is a suitable medium for C. neoformans as long as the pH does not exceed 8 0 which also applies to various media as pointed out previously by Mosberg and Alvarez DeChouderts (22) The relation between

the fungus and the pigeon has however not been cleared up Emmons (5) was unable to isolate C. neoformans from tissue of pigeon whose excreta contained fungi and attempts to infect pigeons experimentally with C. neoformans have so far failed (13 26) The apathogenecity of the fungus for pigeons and other birds is presumably attributable to the high body temperature of the birds, about 41 C (5 26) The sensitivity of C. neoformans to elevated body temperature has previously been described in a number of experimental investigations (e.g 14 16 17)

It is well known that different infections are prone to occur in patients with chronic debilitating diseases. About one third of all cases of cryptococcosis are thus held to occur as a complication in patients with malignant disease of the reticuloendothelial system. No factor definitely responsible for this can be pointed out at present, but the possibility that prolonged steroid treatment in these cases predisposes to the mycotic infection by reducing the host resistance has been discussed (2 10)

Since cryptococcosis occurs only sporadically in Scandinavia and Finland the interest in the infection and the possibility of studying the spread of the disease and the occurrence of C. neoformans in natural sources in these countries are very limited That such a natural source of C. neoformans occurs at least in the southernmost district of Sweden was shown by the present investigation The frequency of positive culture findings is however not in parity with previously published investigations from the U S.A. Nor did the check-examination of autopsy material from a town (Malmö) in south Sweden indicate a high frequency of subclinical cryptococcal infections in association with malignant systemic diseases. Although the results of the investigation were limited the isolation of C. neoformans from pigeon excreta revealed a reservoir of this pathogenic fungus from which human beings may derive infection Until it can be proved that infectivity of cryptococcus-laden pigeon excreta dust for human beings is low it might be advisable to recommend precautions against exposure to C. neoformans.

Summary

Specimens of pigeon excreta were collected and examined for Cryptococcus neoformans. The pigeons came from different localities in the southernmost district of Sweden. C. neoformans confirmed by morphological cultural, biochemical and virulence studies was isolated from 3 of 450 specimens examined. All the three pigeons from whose excreta fungi had been cultured belonged to the same breeder

Re-examination of 185 autopsy cases of leukaemia lymphosarcoma, reticulum cell sarcoma Hodgkin s disease and multiple myeloma revealed no secondary cryptococcal infection.

The results are discussed and precautions are recommended to reduce exposure to infection with C. neoformans.

This is the first time that C. neoformans of non human origin has been demonstrated in Scandinavia.

Acknowledgment

I thank Miss W Slooff, Centraalbureau voor Schimmelcultures, Delft (The Netherlands) for valuable help.

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Pectus Excavatum

By

F. S. P. van Buchem and J. Nieveen

Pectus excavatum (funnel chest) is no rare finding, especially in patients with arachnodactyly (3, 14). When studying the sequelae of this anomaly, it should be realized that there are different degrees of sternal retraction.

In recent years several investigators have expressed their opinion (6, 9, 11, 19) that patients with funnel chest should be treated surgically, even if they have no complaints and no demonstrable heart or lung-function disturbances (9, 19), preferably in the 3rd or 4th year of life. This is called a prophylactic operation. Even *apart from* possible risks or poor operative results, we wonder whether a preventive operation is justified at all.

In order to be able to judge more fully we have subjected eight patients with serious forms of funnel chest to an extensive study.

Clinical data

To give an impression of the degree of the deformity in these patients, fig. 1 shows an anterior view of the thorax of patient no. 1. Fig. 2 gives the lateral thoracic view of this patient, which clearly demonstrates that the distance between the *sternum* and the anterior surface of the spinal column is considerably reduced.

Tables I and II list the complaints and the main clinical data of the patients. Of the eight patients, six were asymptomatic; they came for routine physical examination. One patient (no. 2), aged 60, had once had pain in the chest during a bicycle ride; it passed off after a rest. He had not suffered *from this* pain during the past six months; occasionally he had had palpitations. Patient no. 4 (female) had had palpitations for the past four years. Patients nos 1, 3, 5 and 8 were able to join in sporting activities without any difficulties; patients 6 and 7 were somewhat earlier out of breath during games than their fellow-players. It is a striking fact that only *in patient 7 was a systolic murmur heard*, which, as usual in these patients, was localized left parasternally in the 2nd—3rd intercostal space. The murmur changed in intensity with respiration and disappeared during Valsalva test. In the other patients the heart sounds were normal. The patients had no manifestations of congestive heart failure, no increased venous pressure, no oedema, no enlarged liver and a normal circulation time (8—15 sec. determined with magnesium sulphate). The blood pressure was normal in all of them. Apart from an incomplete right bundle-branch

Submitted for publication May 9, 1963.

Table I. Clinical data in cases of funnel chest

Case	Sex	Age	History	B. P.	ECG	Circ. time (sec.)
1	♂	17	No compl.	130/80	Normal	10
2	♂	60	No compl.	140/100	Normal	15
3	♂	23	No compl.	135/80	I.R.B.B. 0.11 sec.	10
4	♀	36	4 years palpit.	125/80	Normal	8
5	♂	17	No compl.	125/70	Normal	13
6	♀	17	No compl.	105/75	Normal	10
7	o	17	Dyspn. on severe effort	135/90	Normal	12
8	♂	13	No compl.	130/90	Normal	9

Table II. Pulmonary function tests

Case	Age	Height (cm)	Weight (kg)	Sounds	Chest X-ray	Vit. cap.	1 sec. value (%)	Residual air (%)	Art. oxygen saturation (%)
1	17	183	68	Normal	Heart shifted to the left	4,335 (3,830)	80	22.8	100
2	60	177	68	Normal	Heart shifted to the left	3,700 (3 680)	56	33.7	98
4	36	169	47	Normal	Heart shifted to the left	2,400 (3,000)	97	34	
6	17	171	56	Normal	Heart shifted to the left	3 120 (3,400)	93		98
7	17	185	63	Systolic murmur	Normal	4,265 (4,800)	73	24.5	99
8	13	156	43	Normal	Heart shifted to the left	2,850 (2,965)	79	25.5	

Table III. Pressures and O_2 saturation in cases of funnel chest

Case	Sex	V. cava sup.		R. atrium		R. ventr.		Pulm. art.			
								Trunk		Periphery	
		Pressure (mm)	O Sat. (%)	Pressure (mm)	O_2 Sat. (%)	Pressure	O Sat. (%)	Pressure	O Sat. (%)	Pressure (mm)	O_2 Sat. (%)
1	♂	2	86	2	77	28/0	77	28/10	79		100
2	♂	3	75	3	72	20/0	69	20/7	70.9		
4	♀		73.5	3	78			25/10	74	5	99
5	♂	6	71	6	71	26/0	68	30/18	66.5		
6	♀	2	77.5	2	78	16/0	80	16/4	78	8	
7	♂	0	69	0	71.5	22/0	71.5	22/5	71.5	5	99
8	♂	3	78	2	79	26/0	71.5	26/6	72.5		



Fig. 1 Patient no. 1 Anterior view of the thorax.

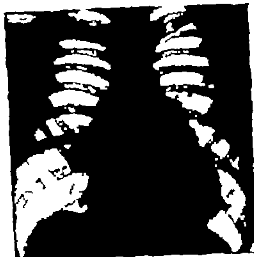


Fig. 3. Patient no. 1 Displacement of the heart to the left with prominent pulmonary arch.



Fig. 2 Patient no 1 Lateral thoracic view

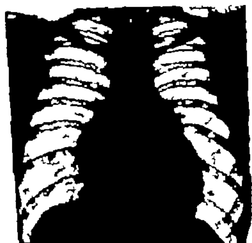


Fig. 4 Patient no. 5 Increased hilar markings.

block, the ECGs showed no abnormalities of any importance

In six patients the X-rays showed displacement of the heart to the left with prominent pulmonary arch, as a result of right axial rotation of the heart (fig. 3). In patient 5 the heart was not displaced, but it was firm with increased hilar markings (fig. 4)

Table III summarizes the data obtained on cardiac catheterization in 7 of the 8 patients. In 6 patients the pressure was normal in the superior vena cava, right atrium, right ventricle and pulmonary artery both in the trunk and, as far as determined, peripherally In the above-discussed patient 5, whose heart was not displaced, the pressure in superior vena

Table I Clinical data in cases of funnel chest

Case	Sex	Age	History	B. P.	ECG	Circ. time (sec.)
1	♂	17	No compl.	130/80	Normal	10
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3	♂	23	No compl.	135/80	I.R.B.B. 0.11 sec.	10
4	♀	36	4 years palpit.	125/80	Normal	8
5	♂	17	No compl.	125/70	Normal	15
6	♀	17	No compl.	105/75	Normal	10
7	♂	17	Dyspn. on severe effort	135/90	Normal	12
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Table II Pulmonary function tests

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2	60	177	68	Normal	Heart shifted to the left	3 700 (3,680)	56	33.7	98
4	36	169	47	Normal	Heart shifted to the left	2,400 (3 000)	97	34	
6	17	171	56	Normal	Heart shifted to the left	3,120 (3,400)	93		96
7	17	185	63	Systolic murmur	Normal	4,265 (4,800)	73	24.5	99
8	13	156	43	Normal	Heart shifted to the left	2,850 (2,963)	79	25.5	

Table III Pressures and O_2 saturation in cases of funnel chest

Case	Sex	V. cava sup.		R. atrium		R. ventr.		Pulm. art.			
								Trunk		Periphery	
		Pressure (mm)	O_2 Sat. (%)	Pressure (mm)	O_2 Sat. (%)	Pressure	O_2 Sat. (%)	Pressure	O_2 Sat. (%)	Pressure (mm)	O_2 Sat. (%)
1	♂	2	86	2	77	28/0	77	28/10	79		100
2	♂	3	75	3	72	20/0	69	20/7	70.9		
4	♀		73.5	3	78			25/10	74	5	99
5	♂	6	71	6	71	26/0	68	30/18	66.5		
6	♀	2	77.5	2	78	16/0	80	16/4	78	8	
7	♂	0	69	0	71.5	22/0	71.5	22/5	71.5	5	99
8	♂	3	78	2	79	26/0	71.5	26/6	72.5		

This picture points to a disturbed right ventricular diastole, which may be caused by the above mentioned affections of the pericardium, myocardium or endocardium, respectively or by funnel chest, as the right ventricle may become compressed between the sternum and the spine. This compression of the right ventricle has been angiocardiographically verified in some cases (7)

In analogy to the situation in constrictive pericarditis, this finding has also become an indication to operation in funnel chest (1 9)

However this picture occurs only in a small minority of cases of funnel chest, including the serious ones Ravitch 1 in 4 Fabricius 2 in 26 Tourniaire 1 in 10 Reusch none in 8 our series 1 in 7 cases. It is true that Bär et al. found it in 5 out of 6 cases, but in 4 of them the end-diastolic pressure amounted to not more than 25 % of the right systolic ventricular pressure. In constrictive pericarditis the end-diastolic pressure is often half or at least one third of the systolic ventricular pressure (2, 19) Here also it is important t mention different degrees of impediment of the right ventricle diastole. Some compression of the right ventricle therefore need not implicate functional disturbance which is, indeed, apparent because in ll cases mentioned no manifestations of congestive heart failure were found (1 7 19 and our patient) Furthermore, on exertion a good adaptation of the cardiac output was demonstrated (7 19)

In older publications emphasis was frequently placed on disturbed lung function in cases of funnel chest. Lester 13) for example reports that the lung volume may be educed by 25 or more, and that the residue is often higher than the normal values. However he does not give more detailed data of volume and frequency As far as more detailed studies have been carried out, and in more serious forms of funnel chest also no or only slight changes of pulmonary function have been found (1 15 19 and our patients) In particular slight limitation of the vital capacity and sometimes mild to moderate (8) increase of the residual air no relationship was found with the degree of the thoracic malformation (1)

ECG often shows details which, however are largely based on displacement and rotation of the heart and the unusual position of the electrodes as a result of the abnormality of the chest wall (10 17 19) These findings are most marked if there is a manifest displacement of the heart, and they often disappeared after correction of the funnel chest. Moreover we are often dealing here with incomplete right bundle-branch block, which not uncommonly (4 18 %) is found in people who otherwise present no indications of a cardiopathy (12)

All things considered, as a rule the sequelae are not too bad as regards heart and lung-function, and also in serious forms of funnel chest. It is often supposed that the condition might become worse with increasing age (11 13) but we did not find any data supporting this contention As we are dealing here with a congenital malformation, it is highly remarkable that congestive heart failure and serious lung function disturbances are only seldom found in adolescents, adults and even in elderly people in whom the affection has already existed for such a long time and often in a serious form (19) In this respect there exists a great difference from constrictive pericarditis, extensive amyloidosis of the heart and endocardial fibro-elastosis, in which, due to the progression of the dis

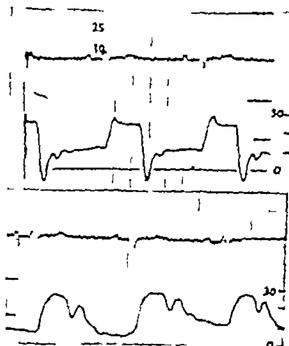


Fig 5 Patient no. 5 Pressure curve of the right ventricle and pulmonary artery

cava and right atrium was somewhat increased (6 mm Hg) The pressure curve of the right ventricle showed a postsystolic dip with raised end-diastolic pressure (13 mm Hg) (fig 5)

Table II lists the most important pulmonary function tests carried out in 6 of the 8 patients.

The vital capacity was somewhat reduced in patient 4 (the woman with the palpitations) and in the two patients (6 and 7) who were somewhat easily dyspnoeic during sports the one second value (Tiffeneau) was as a rule on the high side (73—97 %) The residual values were normal, as was the arterial oxygen saturation, as far as determined (patients 1 2 6 and 7)

Discussion

A survey of the extensive investigations in the recent literature in combination with our own experiences, gives the following facts. Patients with a funnel chest, and particularly if they suffer from the more serious forms, *often have no complaints* and there exists no relationship between the severity of the condition and the possible occurrence of complaints (7) Complaints of palpitations and earlier dyspnoea on particular exertions (sports) are not rarely mentioned.

On physical examination a left parasternal systolic murmur is often heard *This murmur may change in intensity* with the respiration and disappear in Valsalva s test.

In many cases the heart is displaced to the left with right axial rotation which gives rise to a prominent pulmonary arch Manifestations of congestive heart failure have rarely been observed, insofar as no other organic heart changes are present (16) No cases of congestive heart failure have been observed in the publications of recent years (1 7 8, 17 19) and in our patients, although not uncommonly adults, even 52—60 years old and cases of serious forms of funnel chest, are involved.

In most cases (1 7 16 17 19 and in our cases) heart catheterization yielded normal pressures in the superior vena cava, right atrium, right ventricle and pulmonary artery (trunk and lung capillaries) even though sometimes there was a small pressure gradient between right ventricle and pulmonary artery in which however the pressure in the right ventricle was not too high Occasionally the pressure curve of the right ventricle shows a post-systolic dip with elevated end-diastolic pressure (fig 5) in which case the pressure in the superior vena cava and right atrium may be somewhat raised (table III) This picture is seen in a marked form in constrictive pericarditis (2) extensive amyloidosis of the heart (5) and endocardial fibro-elastosis (4) which conditions are, however accompanied by marked congestive manifestations.

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ease signs and symptoms of congestive heart failure become manifest in the long run

In view of the above-mentioned observations we agree with Fabricius et al. (7) Scherf and Boyd (18) Fink et al (8) that in cases of funnel chest operation is only rarely indicated unless considered justified for cosmetic reasons only We therefore disagree with the ideas of Hegemann Buytendijk Tourniaire and Lam et al. that patients with funnel chest should be subjected to operation even if they have no complaints and no demonstrable heart or lung function disturbances. We believe ourselves justified in taking up this position because the above facts have shown that in general the chances of serious sequelae of funnel chest are very small indeed

It is however desirable that follow up studies be made of patients who show the above-mentioned pressure pattern in the right ventricle, or especially if the end diastolic pressure is more than 1/3 of the systolic pressure Six years after the extensive examination there was not a single indication of insufficient heart function in our patient no 5

Finally attention is called to the fact that patients with funnel chest not uncommonly also present manifestations of arachnodactyly not rarely attended by cardiovascular affections of various nature (3 14) for example, medianecrosis cystica of the aorta and pulmonary artery which may explain the development of aneurysms (dissecting) in these patients

McKusick reports that such a patient died of dissecting aneurysm after the surgical correction of funnel chest

Summary

History physical and electrocardiographic manifestations and the results of heart and lung function tests are reported in eight patients with serious forms of funnel chest. Only one patient, in whom the heart was not displaced to the left, showed a pressure gradient in the right ventricle that suggested a limitation of the diastole of the right chamber According to the literature, in these cases the diastolic pressure is usually only one-fourth or barely one-third of the systolic pressure in the right ventricle, which only points to a moderate compression which, as a rule, is not attended by signs and symptoms of disturbed cardiac function.

As the lung function is usually only little disturbed, the authors consider operation to be only rarely indicated and they do not believe a "prophylactic" operation justified.

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From the Institute of Biochemistry (Head G. Ehrensvärd, Ph. D.) University of Lund, and the Medical Department (Head N. Söderström, M. D.)
University Hospital of Lund, Sweden

On Vitamin B_{12} Forms in Human Plasma

By

Kai Lindstrand and Karl-Gustav Ståhlberg

In 1958 Barker et al. (1) demonstrated the occurrence of adenine coenzyme B_{12} in *Clostr. tetanomorfum*. The following year Weissbach et al. (10) found that when these organisms were grown in a medium containing either 5,6-dimethylbenzimidazole or benzimidazole, the bulk of their B_{12} content consisted of 5,6-dimethylbenzimidazole coenzyme (DMBC-coenzyme) B_{12} or benzimidazole coenzyme B_{12}, respectively. In 1961 Toohey and Barker (9) reported observations also suggesting that the DMBC-coenzyme B_{12} represents the main form of B_{12} occurring in the human liver.

These findings prompted us to investigate in what form or forms vitamin B_{12} occurs in human plasma. As far as we know no such investigation has been reported. In addition to substances presumably corresponding to cyanocobalamine, hydroxycobalamine and DMBC-coenzyme B_{12} we found a fourth unidentified factor with vitamin B_{12} activity for *E. coli 113—3* and for *Euglena grac. Z strain*. This substance has not been described before.

Submitted for publication May 14, 1963.

Material and methods

The material consisted of plasma from 8 apparently healthy persons with a serum B_{12} content ranging from 200 to 450 pg/ml.

Extraction of B_{12} from plasma

The extraction was done in a dark room illuminated intermittently with a dim lamp (Philips PF 704 E, dark red). Only laboratory glassware washed in bichromate sulphuric acid was used. Unless otherwise stated, the extraction process was carried out at room temperature.

About 80 ml of venous blood was collected from each person. The blood was allowed to flow directly into test tubes containing sodium citrate and wrapped in aluminium foil. Within a few minutes of collection the blood was centrifuged at 2,000 r.p.m. for 45 min., after which 30 to 40 ml of plasma was pipetted off. Only plasma showing no signs of haemolysis was accepted for B_{12} extraction.

Alcohol extraction

As soon as the plasma had been pipetted off it was poured into absolute alcohol (1 : 4) at + 80° C. The suspension was kept at this temperature for 20 min., chilled in an ice bath and filtered by suction through filter paper. The filtrate was deposited in a rotating evaporator and the alcohol was removed under vacuum at about + 30° C.

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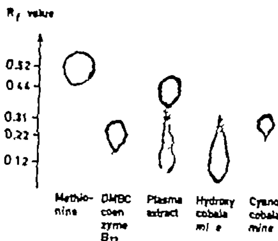


Fig 1 Bioautogram of chromatographed plasma extract and reference substances (traced from photograph) Of the four chromatographic plasma factors with B_1 activity for E. coli 113—3 the one with highest rate of migration gives the most marked growth zone

Ether extraction

The residual water phase, in appearance a milky suspension, was extracted 3 to 6 times with an equal volume of ether Dissolved ether was afterwards removed from the water by evaporation.

Phenol extraction

Phenol containing 15 water was added to the aqueous solution (1 4) and the mixture was shaken in a separating funnel. After the emulsion had been broken down the phenol phase was shaken with 1 part of acetone, 3 parts of ether and a small amount of deionized water The water phase obtained after shaking was washed once with an equal volume of ether to remove the phenol. The ether dissolved in the water was afterwards separated off under vacuum in a rotating evaporator and the water evaporated to 100 μl. The solution was yellow to green in colour and was kept in the dark at + 4 C until chromatographed a few hours later

Chromatography of plasma extract dissolved in water

Ascending chromatography was done in the dark with Whatman No. 2 paper (26 cm × 26 cm) in sec. Butanol — conc. acetic acid — water (100/3/50) for 16 to 18 hours.
Reference substances

DL-Methionine supplied by L. Light and Co. Ltd. England.

Cyanocobalamine A pharmaceutical preparation, Ido B_{12} supplied by AB Ferrosan, Malmö, was purified by elution with deionized water from a CM cellulose column (Whatman powder CM 70) at pH 5.5 After paper chromatography of the eluate bioautography showed only one distinct zone of growth with an R_f value corresponding to cyanocobalamine (3)

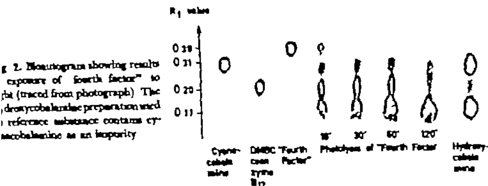
Hydroxycobalamine Oxocobemin was supplied by AB Vitrum, Stockholm For removal of contaminants a visible amount of Oxocobemin was added to a Dowex 50 W column at pH 3 With a sodium acetate buffer of increasing molarity and pH, contaminants such as cyanocobalamine were removed. The hydroxycobalamine was eluated with 0.2 M sodium acetate buffer at pH 8. After elution the eluate was concentrated and the salts were extracted with phenol After redistribution to the aqueous solution, the latter was chromatographed.

Bioautography of the chromatogram showed the presence of mainly hydroxycobalamine (R_f value according to Barker et al. (2)) but also smaller amounts of cyanocobalamine.

5,6-dimethylbenzimidazole (DMBC) — coenzyme B_{12} obtained from Dr D Perlman, the Squibb Institute for Medical Research, New Brunswick, N.J., was purified on a Dowex 50 W column at pH 3 The column was washed with sodium acetate of increasing molarity and pH. DMBC-coenzyme B_{12} was eluted with 0.030 M sodium acetate buffer at pH 5.5—6 2 (2) The eluate was extracted with phenol. After redistribution to the aqueous solution chromatography was done and later bioautography Only one distinct zone of growth with an R_f value corresponding to that given for DMBC-coenzyme was demonstrable (2)

Microbiological examination

a) *Bioautography E coli* 113—3 (ATCC 10586) was used as a test organism. Growth of this strain requires B_1 which can, however be replaced by methionine in higher concentration (10,000—30,000 fold) (5) Stock cultures of the bacteria in nutrient agar (Bacto Beef Extract 3 g Bacto Peptone 5 g, NaCl 5 g, Agar 18 g, Aq dest. ad 1 000 g)



...g. 2. Bioautogram showing results ... exposure of "fourth factor" to ...ght (traced from photograph). The ...hydroxycobalamine preparation used ... reference substance contains cy-...anocobalamine as an impurity

were incubated for 24 hours at + 37° C and then kept at + 4 C. Inocula were prepared by transferring bacteria from the stock culture to Bacto Micro Inoculum Broth and incubating the suspension at + 37° C for 24 hours. After incubation the bacteria were washed three times with saline. The density of the cell suspension was determined turbidimetrically in Junior Spectrophotometer Model 6 A (Coleman Instruments Inc., U.S.A.) and adjusted to 40 % T. Within one hour from the beginning of the washing procedure the bacteria were inoculated in sterile medium consisting of 7 g of K_2HPO_4, 3 g of KH_2PO_4, 0.59 g of sodium citrate ($2\ H_2O$), 0.19 g of $MgSO_4$ ($7\ H_2O$), 1.09 g of $(NH_4)_2SO_4$, 18 g of Difco Special Agar Noble, 10 g of glucose per 1,000 g of water.

The medium containing bacteria in even suspension was poured immediately into square glass dishes (30 cm × 30 cm). After 4 hours preincubation of the agar dishes at + 37 C the chromatograms were placed on the agar and left there for 16 hours at + 37° C. The chromatograms were then removed, and the bioautograms were read and photographed or traced.

b. *Determination of vitamin B_{12} activity with Euglena grac. Z strain according to Killander* (7). The B_{12} content of the normal sera was determined by this method.

The B_{12} activity of an unidentified substance ("fourth factor") found in chromatographically purified plasma extract and stimulating the growth of *E. coli* 113—3 was studied qualitatively with the *Euglena grac.* test.

Results

In all 8 cases studied bioautography of the chromatograms of plasma extracts showed four growth zones (fig. 1). Of these zones, one had an R_f value on the chromatogram corresponding to cyanocobalamine, one to hydroxycobalamine, one to the DMBC-coenzyme B_{12}. The fourth zone, which contained a factor stimulating the growth of *E. coli* 113—3 did not correspond to any of the reference substances used for chromatography. It was invariably the most marked zone of growth. The unknown factor corresponding to this fourth growth zone proved to possess vitamin B_{12} activity also for *Euglena gracilis Z* strain.

For further examination an aqueous solution of the chromatographically purified fourth factor was divided into four 5 µl aliquots. The aliquots were then exposed to illumination by a 40 W lamp for 10, 30, 60 and 120 minutes, respectively. The distance between the aliquots and the lamp was 40 cm. The effect of the exposure was checked chromatographically and with subsequent bioautography (fig. 2). Ten minutes illumination of the factor produced a small fourth zone of growth, faint growth zones corresponding to R_f values

for cyanocobalamine and hydroxycobal amine and a marked zone with the R_f value of DMBC-coenzyme B_{12}. After 30 minutes exposure to light the fourth zone was no longer visible. The other zones persisted and of these the zone corresponding to DMBC-coenzyme was the most marked On illumination for 120 minutes only one marked zone with an R_f value corresponding to hydroxycobal amine was demonstrable.

Discussion

The main purpose of the investigation was to check whether DMBC-coenzyme B_{12} is a major component of the plasma B_{12}. The chemical and physical properties of the DMBC-coenzyme are fairly well known (4) Structurally the coenzyme differs from cyanocobalamine by the fact that the cyanogroup linked to the cobalt atom is replaced by a nucleoside. It is labile and readily decomposed by light with the formation of hydroxycobalamine and adenine nucleoside. Therefore only fresh plasma was used and it was analysed as soon as possible and in the dark

Bioautographic analysis of the chromatogram revealed three plasma factors with B_{12} activity for *E coli* 113—3 and R_f values corresponding to DMBC-coenzyme, cyanocobalamine and hydroxycobal amine, respectively as well as a fourth factor capable of stimulating the growth of *E. coli* 113—3 Since this factor was found to possess B_{12} activity also for *Euglena grac Z* strain it is probable that it constitutes a complete B_{12} analogue (6) On bioautography it appeared as the predominant B_{12} component of the plasma. It proved extremely sensitive to light On exposure to light it was converted via some substance, presumably DMBC-coenzyme B_{12}, to a substance which was probably hydroxycobalamine. This light induced degradation was accompanied by the appearance of another substance, presumably cyanocobalamine.

Owing to the minute B_{12} content of the plasma it was not possible to identify the fourth substance chemically However we have recently been able to demonstrate the presence of the fourth factor in hog liver in amounts sufficient for attempting identification (to be published)

In view of the hitherto demonstrated properties of the fourth factor it does not seem unreasonable to suppose that it might be a physiological form of vitamin B_{12}. But for the time being the possibility of this fourth factor being an artefact of the extraction of the plasma and liver cannot be excluded

Summary

Bioautographic analysis of a chromatographed extract of normal human plasma revealed four factors with vitamin B_{12} activity for *E coli* 113—3 Of these, three were presumably cyanocobalamine, hydroxycobalamine and DMBC-coenzyme B_{12}. The fourth factor which has not yet been identified has activity for *Euglena gracilis Z* strain and on photolysis appears to be converted into a substance which is probably DMBC-coenzyme B_{12} which on further photolysis presumably is converted into hydroxycobalamine.

Acknowledgements

The authors are greatly indebted to Dr D. Perlman for generous gift of DMBC-coenzyme B_{12}. We also want to thank Drs. Z. Banhidi and H. Jylling for kind and most valuable advice on the bioautographic technique.

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The Peripheral Blood Flow in Intermittent Claudication

VI. Plethysmographic Studies. The Blood Flow Response to Exercise with Arrested and with Free Circulation

By

Leif K. Hillestad

The mechanical effect of muscle contraction upon the muscle blood flow is known from a series of notable investigations (2, 3, 4, 5, 6, 30). So is also the circulatory response to exercise of the skeletal muscles of normal subjects (9, 15, 21, 28). Except for certain useful contributions (29, 33, 34) similar data are scarce for conditions where the blood supply to the muscles is restricted by obliterative arterial disease.

The purpose of the present paper is to widen our knowledge on this subject.

This has been done by evaluating the flow response to rhythmic exercise of the calf muscles in normal limbs and limbs with intermittent claudication of the calf due to main artery obstructions. A basis has thereby been established for the use of exercise tests in clinical examinations of the peripheral blood flow.

The calf blood flow has been assessed by means of venous occlusion plethysmography, the reliability of which has been further strengthened by recent investigations (12).

In the following the flow response of the calf to exercise with arrested circulation (ischemic exercise) and with free circulation (free exercise) is described. The combined use of ischemic and free exercise in flow studies and some associated problems will be dealt with in a later communication.

Material

The healthy subjects were aged from 21 to 68 years. The patients were aged from 38 to 63 years and suffered all from intermittent claudication of the calf due to obliterative arteriosclerosis of the main arteries. In none of them was any other disease detectable, and in none were notable trophic lesions of the limbs demonstrable. All the patients were admitted to the hospital and went through the routine examinations including arteriography. The prevalent site of the obstructive lesion was the femoral artery, but some cases with obstructions located at the aorto-iliac or the popliteal level of the main artery were also included.

In the majority of the patients the main artery stenosis was complete, and the associated collateral circulation showed a different development from case to case.

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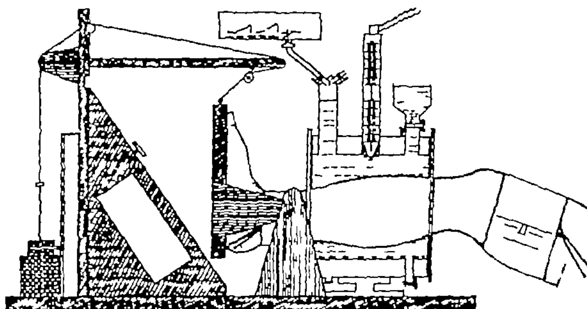


Fig 1 The arrangement of the experiments with the ergograph connected to the plethysmograph.

Methods

Exercise plethysmography

For the conduction of plethysmography and the use of the data obtained, reference is made to the previous papers of this series.

In order to introduce exercise an ergograph was connected to the plethysmograph (fig 1) The foot board was arranged so that the axis of its movement passed through an imaginary axis of the ankle joint as in walking (33) With the leg kept horizontal the pedalling had therefore to be done mainly by the posterior calf muscles. When the foot board was depressed until it stopped against a metal knob, the load of 4.5 kg was lifted 21 cm. At this point the calf muscles were immediately relaxed, and the descent of the load moved the foot-board back into its original position. Alternate contraction and relaxation of the muscles was thereby obtained. The exercise was timed with a metronome at a rate of thirty contractions per min.

During ischemic exercise the circulation was kept arrested by a sufficient inflation of the proximal cuff.

When the first 5 min. of the hyperemia had passed, the distal cuff was deflated for a little while in order to avoid ischemic pain in the foot. The measurements were not started again until the foot flow had again been arrested for 1 min. This procedure had to be repeated for several times during the recordings of the prolonged pathologic hyperemias.

In performing the exercise as described above most of the patients will experience pain after a time somewhere between 1 and 2 min For the present study only those patients were selected who could manage exercise for 1 1/2 min. without pain and who could also carry out the 2 min. exercise properly Most of the patients had uncomfortable pain of the calf during the last period of the 2 min. exercise.

Some technical difficulties were encountered in this work. During the huge flows following ischemic exercise in normal subjects an overflow of water into the transmission system may easily occur This had to be prevented by keeping the water level of the recording tube somewhat lower than usual. Similarly the water level had to be watched carefully during the free exercise. The calf volume then decreased considerably because the veins were emptied by means of the muscle pump. The water level could not be allowed to descend into the plethysmograph, since even the most rapid refilling would not be a guarantee against formation of air bubbles in the apparatus. After the cessation of free exercise the calf volume increased rapidly due to filling of the veins. Again the water level had to be

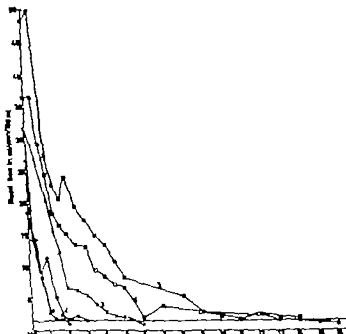


Fig. 2. The hyperemias of normal calf following ischemic exercise of the calf muscles for 15 sec. (1) 30 sec. (2) 60 sec. (3), 90 sec. (4) and 120 sec. (5)

adjusted steadily in order to prevent over-flow of water as described above

A further difficulty encountered in exercise plethysmography is the change of the resting flow during the experiments, phenomenon also noted by Grant (20) This adds to the trouble met with in measuring the prolonged pathologic hyperemias. These often subsid so slowly tha the slope for long distances may be nearly horizontal, and it is not easy to decide then whether new level of the resting flow has occurred or whether hyperemia is still present However usuall the hyperemia can be assessed without material errors

The exercise was followed by transient, but insignifican rise of the systemic blood pressur in some of he patients No ppreciable rise of he cardiac rate was observed.

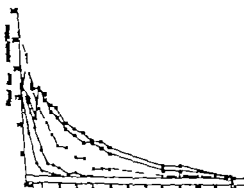


Fig. 3 The hyperemias of the calf in limb with intermittent claudication of the calf due to an incomplete main artery stenosis. The experiment was carried out and the curves are marked for identification as explained in the legend to fig 2.

Results

The blood flow response of the calf to ischemic xercise

When the skeletal muscle with normal blood supply is exposed to increasing amounts of ischemic exercise, there occurs a rapid rise of its post-contraction hyperemia (fig 2) This maintains a typical shape throughout the experiment with a great initial flow which at first

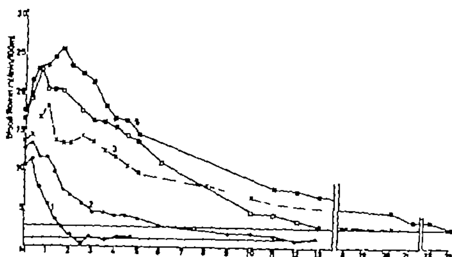


Fig 4 The hyperemias of the calf in a limb with intermittent claudication of the calf due to a complete main artery stenosis. Excellent collateral circulation The experiment was carried out and the curves are marked for identification as explained in the legend to fig 2

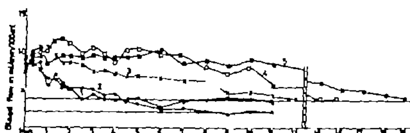


Fig 5. The hyperemias of the calf in a limb with intermittent claudication of the calf due to a complete main artery stenosis. Poor collateral circulation The experiment was carried out and the curves are marked for identification as explained in the legend to fig

subsides quickly and later on slowly until the resting flow is attained (1 33)

If the main artery of the skeletal muscle is the seat of an incomplete stenosis the same experiment leads to a different result (fig 3) Still more marked are the changes when the main artery is completely occluded The picture is then somewhat different in the presence of an excellent (fig 4) and of a poor (fig 5) collateral supply In cases with still more advanced disease the hyperemia may initially be lower than the resting flow as reported by Shepherd (33) and as also demonstrated previously in this series.

The changes taking place during the post-exercise hyperemia in advancing obliterative disease can now be summarized The peak flow becomes successively lower and more and more delayed. It increases less than normal with increasing amounts of exercise. The hyperemia curve becomes prolonged and of a protracted shape The initial loss of flow is compensated by a longer duration of the hyperemia.

The observed changes may be taken to favor a debt theory and it is crucial to know whether or not a definite amount of ischemic exercise produces an identical

amount of hyperemic blood flow regardless of the patency of the main arterial blood supply. The following experiments were carried out to get an answer to this problem.

The magnitude of the blood flow response is observed to increase with increasing extents of ischemic exercise, as expected (fig. 6). The shortest exercise is of 15 sec. duration and thus represents a very small amount of work.

A study of the normal flow response allows the conclusion that it is linearly related to the ischemic exercise provided this is of an extent similar to or greater than one min. If the slope of this part of the response is prolonged downwards, it will cut the abscissa at about the point of 45 sec. This could be taken to indicate that for periods of exercise below 45 sec. the flow response would be virtually zero. As the diagram shows, this is not the case. On the contrary there is a response, and this is greater than according to the slope for the bigger responses. Moreover the flow response to small amounts of ischemic work points directly towards origo. These findings are at variance with those of McArdle and Verel (28).

The pathologic flow response differs from the normal one in being linearly related to the ischemic exercise right from the start. Moreover the pathologic response is greater for each amount of exercise until that lasting for 2 min. However the difference decreases with increasing amount of exercise.

The relative fall of the pathologic response following 2 min. of ischemic exercise is by no means incidental. A majority of the patients experienced claudication towards the end of this amount of exercise. In the limbs with less

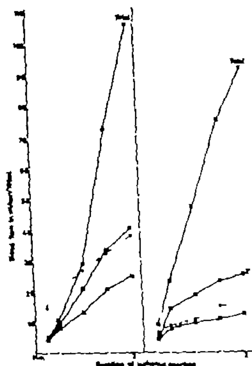


Fig. 6. The blood flow response of the calf to increasing amounts of ischemic exercise of the calf muscles in normal limbs (left side) and in limbs with intermittent claudication of the calf due to femoral artery stenosis (right side). The total hyperemia (Total) divided by the flow during the first min. (1) and during the second min. (2). The peak flow represented by an interrupted line. Mean values from examination of five normal limbs and eight limbs with intermittent claudication.

collateral circulation the response became of a normal magnitude. In the other limbs the response was invariably less, and in a few cases the response was only a little greater than that following exercise for one and a half min. The conclusion is warranted that when the claudication comes on the relationship between the pathologic flow response and the amount of ischemic exercise breaks

Table I The magnitude of the post-contraction hyperemia of the calf in response to 1 min of ischemic exercise of the calf muscles. Mean values from 32 examinations of 22 normal subjects The hyperemia (excess blood flow) is divided into portions corresponding to the flow during the first min of the second min. of the third and fourth and fifth min of and the rest of the hyperemia

	Excess blood flow (ml/min/100 ml)					
	1st min	2nd min	3rd—5th min	Rest	Total	Peak
Mean	13.1	4 7	4.3	0.4	21 7	29.8
S.D	2.4	2.3	3.0	0.8	6.8	6.0
Range	8 0—15.0	1.5—9.5	0.2—9.8	0.0—3.5	14.0—36.3	18.5—42.0

Table II The magnitude of the post-contraction hyperemia of the calf in response to 1 min. of ischemic exercise of the calf muscles. Mean values from 16 limbs with intermittent claudication of the calf due to incomplete or complete stenosis of the main artery and with varying extents of ollateral circulation The hyperemia is divided into portions as in table I

	Excess blood flow (ml/min/100 ml)					
	1st min	2nd min	3rd—5th min	Rest	Total	Peak
Mean	8.1	7 1	12.6	10.8	38.6	12.4
S.D	3.9	3 1	4.5	8.3	10.7	5.5
Range	2.0—18.9	2.0—11.8	2.0—21.2	3.5—33.5	14 0—75.2	6.0—28.9

On superficial examination of the diagram it is tempting to draw a straight slope through all the points and conclude that both the normal and the pathologic responses are linearly related throughout to the amount of exercise. The demonstration of irregular responses at both ends of the range of work proves that this would be erroneous.

If the behaviour of the peak flow and the various divisions of the hyperemia is observed the abnormal shape of the pathologic hyperemia is clearly evident.

An increase of the stimulus i e the exercise, produces an increased flow in the normal as well as in the pathologic circulatory system. But however much the stimulus is increased the qualitative and quantitative differences between the two systems are maintained. The abnormality of the pathologic system becomes indeed more pronounced It is obvious therefore that the increased flow following increased stimulation is not consistent with an improvement of the circulation In pathology an improvement of a diseased organ means a change of its function towards the normal function of that particular organ Consequently an improved flow through the pathologic system can come about only by a change of its flow pattern towards the normal one in response to a definite amount of exercise

From this point of view an improved flow through the diseased limbs can only occur *ceteris paribus* by the following changes of the post-exercise hyperemia An increase of the peak flow a reduction of the magnitude and a normalization of

Table III The post-contraction hyperemia of the calf following 1 min. of ischemic exercise of the calf muscles before and after successful reconstructive arterial surgery in 13 limbs with intermittent claudication of the calf

	Excess blood flow (ml/min/100 ml)					
	1st min	2nd min	3rd–5th min	Rest	Total	Peak
After op.	14.9	8.3	10.8	5.5	39.5	25.3
Before op.	5.6	5.2	10.3	9.2	30.3	9.6

Table IV The ratio of the first minute flow to the remaining part of the hyperemia of the calf following 1 min. of ischemic exercise (A) The ratio of the first minute flow to the flow during the second, third, fourth and fifth min. of the same hyperemia (B)

	Flow ratios	
	A	B
Normal limbs (table I)	1.40	1.45
Pathol limbs (table II)	0.27	0.41
Op. limbs (table III)	0.61	0.78
Non-op. limbs (table III)	0.23	0.56

the shape The latter requirement implies that the first min. flow must count for a greater share of the total hyperemia.

It must now be taken into account that in the treatment of obliterative disease there is never a complete cure. Even modern surgery is not able to remove more than the gross arterial changes, and still less is to be expected by means of the available drugs It is therefore important to control the above requirements for an improved circulation by applying an ischemic exercise test upon a clinical series.

For this purpose a test consisting of 1 min of ischemic exercise seems to be

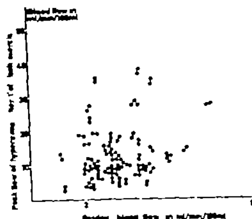


Fig 7 The distribution of the resting blood flow and the peak flow of the hyperemia of the calf in a number of normal limbs (open circles) and limbs with intermittent claudication of the calf due to main artery stenosis (closed circles)

suitable. Such an amount of anaerobic work can be carried out by all patients with intermittent claudication without discomfort. In selecting 1 min. for the test it is furthermore possible to avoid the irregular responses mentioned earlier

The outcome of the test in a series of normal and diseased limbs is as might be expected (table I and II) The spread of the normal values is naturally great and due to the many individual differences, such as training (15) The corresponding spread of the pathologic values is due to the varying arterial involvement of the limbs examined.

However in assessing the great improvement following reconstructive surgery the above requirements for an improved flow are not completely satisfied (table III) The peak flow is certainly increased as is also the first min. flow and its ratio to the rest of the hyperemia, but the hyperemia is not quantitatively reduced. On the contrary it has increased a little, and it must be emphasized that

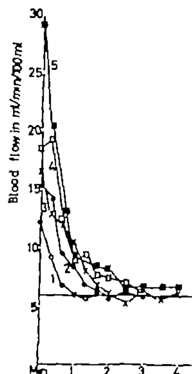


Fig 8 The hyperemias of a normal calf following free exercise of the calf muscles for 15 sec. (1) 30 sec. (2) 60 sec. (3) 90 sec. (4) and 120 sec. (5)

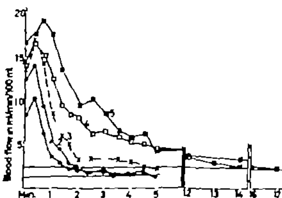


Fig 9 The hyperemias f the calf in a limb with intermittent claudication f the calf due to an incomplet main artery stenosis. The experiment was carried t and the curves are marked as explained in the legend t fig 8

such an increase took place in seven of the thirteen limbs examined This finding is important for the practical use of the ischemic exercise test and shows that purely experimental observations may sometimes have to be modified. Moreover it is not necessary to be too much concerned with the absolute size of the hyperemia, because an improvement can be distinguished from a vasodilatation by assessing the ratio of the first min. flow to the remaining part of the hyperemia. With an improvement this ratio increases while it decreases with vasodilatation due to increased stimulation (fig 6)

Table I The spontaneous variation of the hyperemia following 1 min. of ischemic exercise Mean values calculated from the calf flow of 10 patients with intermittent claudication, whose symptoms remained stable for the 4 months of observation. The blood flow was examined once a month

	Mean individual variation *'	Variation of the group %
Peak flow	13.6	4.6
1st min flow	12.3	2.6
2nd min flow	11 1	3.6
5th min flow	15.7	5.6

Since the total hyperemia is of little use it is of interest to find out whether it would be sufficient to register only the first part of it. This would shorten the time consumed by the examination and save the patient from the discomfort connected with recording of the prolonged pathologic hyperemia It appears that the same certainty of the measurements can be obtained by using only the first 5 min of the hyperemia (table IV) The ratios are thereby somewhat increased but the relationship between them in respect of improvement is maintained

The peak flow of the hyperemia following 1 min of ischemic exercise offers a reliable basis for classification of the blood supply to the limb (fig 7) The distribution of the flows, their magnitudes and the borderline between normal and

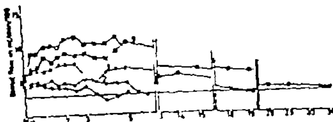


Fig 10. The hyperemias of the calf in limb with intermittent claudication of the calf due to a complete main artery stenosis. The experiment was carried out and the curves are marked as explained in the legend to fig. 8.

pathologic values are similar to those obtained by means of the peak flow following 5 min. of circulatory arrest.

In applying the ischemic exercise test in clinical studies, knowledge of the spontaneous variation of the results is essential (table V). The individual variation is great, but is within the range usually encountered in physiology. Already by examination of a group of ten patients the variation becomes considerably reduced so that the test is obviously serviceable in long-term investigations.

The blood flow response of the calf to free exercise

In rhythmic exercise with free circulation the blood flow is actually stopped by the muscle contractions, whereas the blood streams rapidly through the dilated arteries of the muscle between the contractions (2, 3, 4, 5, 6). In a healthy circulation a great amount of blood will therefore pass through the muscle during the rhythmic exercise, and there will be little post-exercise hyperemia (fig. 8).

When the blood flow to the muscle is restricted by obliterative disease of the main arteries, the corresponding flow during the exercise will be subnormal. In order to satisfy the requirement for blood the post-exercise hyperemia has to increase (fig. 9 and 10). Again the typical pattern of the pathologic hyperemia are

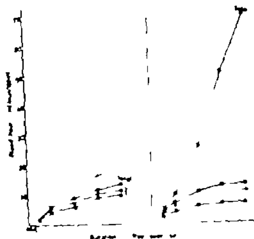


Fig. 11. The blood flow response of the calf to increasing amounts of free exercise of the calf muscles in normal limbs, left side, and in limbs with intermittent claudication of the calf due to main artery stenosis, right side. The total hyperemia (Total) divided by the flow during the first min. (1) and during the second min. (2). The peak flow represented by an interrupted line. Mean values from examination of five normal limbs and twelve limbs with intermittent claudication.

observed with the subnormal and delayed peak flow and the protracted course. The most striking feature is however the large increase of the magnitude of the hyperemia with restricted blood supply. It is therefore reasonable to regard the degree of the hyperemia following free exercise as an index of the actual blood supply.

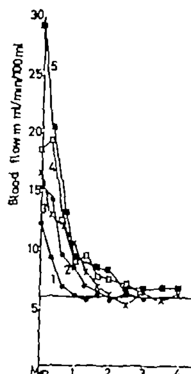


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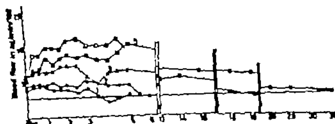
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2nd min flow	11 1	3.6
5th min flow	15 7	3.6

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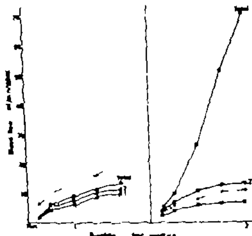


Fig. 11. The blood flow response of the calf to increasing amounts of free exercise: I the calf muscles in normal limbs (left side) and in limbs with intermittent claudication of the calf due to main artery stenosis (right side). The total hyperemia (Total) divided by the flow during the first min. (1') and during the second min. (2'). The peak flow represented by an interrupted line. Mean values from examination of five normal limbs and twelve limbs with intermittent claudication.

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When the blood flow to the muscle is restricted by obliterative disease of the main arteries, the corresponding flow during the exercise will be subnormal. In order to satisfy the requirement for blood the post-exercise hyperemia has to increase (fig. 9 and 10). Again the typical patterns of the pathologic hyperemia are observed with the subnormal and delayed peak flow and the protracted course. The most striking feature is, however, the large increase of the magnitude of the hyperemia with restricted blood supply. It is therefore reasonable to regard the degree of the hyperemia following free exercise as an index of the actual blood supply.

Table VI The magnitude of the post-contraction hyperemia of the calf in response to 1 min. of free exercise of the calf muscles Mean values from 32 examinations of 22 normal subjects. The hyperemia (the excess blood flow) is divided into portions corresponding to the flow during the first min., second min., third and fourth and fifth min. and the rest

	Excess blood flow (ml/min/100 ml)					
	1st min	2nd min	3rd—5th min	Rest	Total	Peak
Mean	5.1	1 1	0.8	0.0	7 0	12.0
S.D	2.2	0.8	1.0	0.0	3.5	3.8
Range	1.3–9.8	0.0–2.5	0.0–3.2	—	1.5–12.7	4.5–15.5

Table VII The magnitude of the post-contraction hyperemia of the calf in response to 1 min. of free exercise of the calf muscles Mean values from 16 limbs with intermittent claudication of the calf due to main artery stenosis. There was considerable variation in the extent of stenosis and development of collaterals

	Excess blood flow (ml/min/100 ml)					
	1st min	2nd min	3rd—5th min	Rest	Total	Peak
Mean	6.7	4.6	7 0	2.3	20.6	9.4
S.D	1.3	1 7	2 6	2.9	6.9	3.2
Range	5.7–10.3	3.3–10.8	2.5–12.0	0.0–10.2	13.2–41.3	7 5–14.8

Evidence supporting this assumption was obtained by recording the flow response to increasing amounts of free exercise in a series of normal and diseased limbs (fig 11) The normal response is small and related in a linear manner to the amount of work provided that the exercise is made for 30 sec or more For smaller amounts of exercise there is an absolute as well as a relative decrease of the response and it appears likely that for exercise below 15 sec the response would actually be zero This means that for small amounts of free exercise the blood flow during the exercise might be sufficient to satisfy the requirement.

The peak flows have a course which runs parallel to the total responses but a little higher They seem also to decrease more rapidly when the exercise is performed for less than 30 sec. but not so rapidly that their slope will cut the abscissa. This indicates that even small amounts of work will produce an increased peak flow even if there is no demonstrable post-exercise hyperemia. The peak flow must then be taken as a sign of vasodilatation during the work. These findings are in accordance with those of Black (9) and Halliday (21)

The pathologic response is markedly different from the normal one. It is larger right from the start of the experiment and increases very fast and in an essentially linear manner with the amount of work done. The diagram provides a useful illustration of the great sensitivity of the pathological circulation to exercise.

It can be seen that the response to 2 min. of exercise is a little less than according to the regression line of the other responses. The relative diminution of the response is, however not so pronounced

as for the corresponding amount of ischemic exercise (fig 6) Maybe this is in part due to the fact that the claudication was not so marked during the free exercise as during the ischemic. It is, however to be stressed that in several of the diseased limbs there was a distinct decrease of the flow response when the exercise was great enough to precipitate ischemic pain. In a few cases the response to 2 min. of exercise was even less than that to 1 1/2 min of exercise. In others the response to 2 min. was only a little higher than that to 1 1/2 min. In some limbs with incomplete stenosis or with complete stenosis and an excellent collateral flow the responses were so great as to match those following the shorter periods of exercise.

Study of the diagram (fig 11) brings up again the earlier comments about vasodilatation and improvement. This time an improvement will require a definite but not marked increase of the peak flow while the increase of the first min. flow may be insignificant. The magnitude of the hyperemia has to decrease, and its shape has to be normalized. The ratio of the first min. flow to the rest of the hyperemia must increase. It is obvious that this ratio will decrease through a vasodilatation incurred by augmented stimulation.

These considerations are largely supported by comparison of the responses in normal and a pathologic series (table VI and VII) The normal post-exercise flow is significantly less than and passes off faster than the pathologic one However the differences of the peak flows and the first min. flows are not significant, and it is useful to look at the corresponding values produced by the ischemic exercise test (table I and II) The conclusion then becomes clear that, in classifying the blood supply the peak flow produced

Table VIII The post-contraction hyperemia of the calf following 1 min. of free exercise of the calf muscles before and after successful reconstructive arterial surgery in 13 limbs with intermittent claudication of the calf

	Excess blood flow (ml/min/100 ml)					
	1st min	2nd min	3rd—5th min	Rest	Total	Peak
After op.	6.5	3.2	4.2	1.0	14.9	11.5
Before op.	5.2	4.4	6.3	3.5	21.4	8.5

Table IX The ratio of the first minute flow to the remaining flow of the total hyperemia following 1 min. of free exercise (A). The ratio of the first minute flow to the flow during the second, third, fourth and fifth min. of the hyperemia following 1 min. of free exercise (B)

	Flow ratios	
	A	B
Normal limbs (table VI)	2.70	2.70
Pathol. limbs (table VII)	0.49	0.58
Operated limbs (table VIII)	0.77	0.88
Non-op.-limbs (table VIII)	0.32	0.49

by the free exercise test is of little or no use in contrast with the peak flow produced by the ischemic exercise test.

An estimation of the changes obtained by reconstructive surgery reinforces the above evidence (table VIII) The improvement obtained by the operation is much better illustrated by means of the ischemic exercise test (table III) Again it will be observed that the operated limbs have not become quite normal, because the hyperemia in response to 1 min. of free exercise is still twice the normal value.

Also for this free exercise hyperemia would be an advantage if it were sufficient to measure it only for the first 5 min

Table VI The magnitude of the post-contraction hyperemia of the calf in response to 1 min. of free exercise of the calf muscles. Mean values from 32 examinations of 22 normal subjects. The hyperemia (the excess blood flow) is divided into portions corresponding to the flow during the first min., second min., third and fourth and fifth min. and the rest

	Excess blood flow (ml/min/100 ml)					
	1st min	2nd min	3rd—5th min	Rest	Total	Peak
Mean	5.1	1.1	0.8	0.0	7.0	12.0
S.D.	2.2	0.8	1.0	0.0	3.5	3.8
Range	1.5—9.8	0.0—2.5	0.0—3.2	—	1.5—12.7	4.5—13.5

Table VII The magnitude of the post-contraction hyperemia of the calf in response to 1 min. of free exercise of the calf muscles. Mean values from 16 limbs with intermittent claudication of the calf due to main artery stenosis. There was considerable variation in the extent of stenosis and development of collaterals

	Excess blood flow (ml/min/100 ml)					
	1st min	2nd min	3rd—5th min	Rest	Total	Peak
Mean	6.7	4.6	7.0	2.3	20.6	9.4
S.D	1.3	1.7	6	2.9	6.9	3.2
Range	5.7—10.3	3.3—10.8	2.5—12.0	0.0—10.2	13.2—41.3	7.3—14.8

Evidence supporting this assumption was obtained by recording the flow response to increasing amounts of free exercise in a series of normal and diseased limbs (fig. 11). The normal response is small and related in a linear manner to the amount of work provided that the exercise is made for 30 sec. or more. For smaller amounts of exercise there is an absolute as well as a relative decrease of the response, and it appears likely that for exercise below 15 sec. the response would actually be zero. This means that for small amounts of free exercise the blood flow during the exercise might be sufficient to satisfy the requirement.

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It can be seen that the response to 2 min. of exercise is a little less than according to the regression line of the other responses. The relative diminution of the response is, however, not so pronounced

Unfortunately the mechanism responsible for the vasodilatation occurring in response to muscular exercise is unknown. It seems that a stable metabolite (17 21) is released by the contractile process, but oxygen lack (25 27) lactic acid (32) pH changes (15, 19) carbon dioxide (26) acetylcholine (22) histamine (14) and adenosine compounds can be excluded. The significance of the potassium ion is moreover uncertain in this respect (10, 24)

Only one conclusion is justified from the present study of the blood flow response to ischemic exercise. This conclusion is that provided a metabolite is responsible, the removal of this metabolite is not critically dependent on the actual rate of flow

In this connection it must not be forgotten that a non-metabolic mechanism also has an influence on the post-exercise hyperemia. Under normal circumstances exercise of the skeletal muscle is regularly associated with dilation of the larger extra-muscular arteries of the limb (23) It is probable that this large-artery dilation will be subnormal or absent when these arteries become the seat of structural changes due to obliterative disease

In their study of the normal circulatory response to ischemic exercise, McArdle and Verel (28) suggested that the debt produced by small amounts of work could be met by oxygen released from local sources such as trapped red cells and myoglobin These small amounts of work would therefore not be followed by any post-exercise hyperemia. Their suggestion is not supported by the present report as the response to small amounts of work was greater than according to the trend of the other responses. Also from a theoretical standpoint their suggestion is difficult to accept, for the reason that an ischemic period is included in the ischemic exercise. Short periods of ischemia are followed by a hyperemia produced by the intraarterial pressure fall (7) and this pressure fall is not dependent on the available amount of oxygen

However as is demonstrated in the present report, small amounts of free exercise may possibly not be followed by a hyperemia because the debt may be satisfied by the blood flow during the work. The finding that the post-exercise hyperemia in response to free exercise increased with obliterative disease is in contrast to the results reported by Dornhorst and Whelan (13) By an acute reduction of the local effective pressure in normal limbs they observed an overall diminution of the post-exercise flows. It seems impossible, however to apply this result to the pathologic limbs, in which the possible reduction of the pressure has been present for years.

The second item emanating from the present analysis is the problem of vascular spasm, recently re-emphasized by the demonstration of spasms in the coronary arteries (18)

Finally the results from a previous paper of this series on claudication should be recalled. It was shown that in extending the exercise from the point of the very first pain to the point of intolerable claudication the gain in terms of blood flow was rather small. However there actually was a gain or in other words an increase of the post-exercise hyperemia. Consequently it had to be concluded that claudication was not associated with vascular spasms.

This conclusion is substantiated by the results of this report.

Still the question remains why the increase of the hyperemia becomes less than

Table X. The spontaneous variation of the hyperemia of the calf following 1 min. of free exercise. Mean values calculated from the calf flow of ten patients with intermittent claudication of the calf whose symptoms remained stable for the 4 months of observation. The blood flow was examined once a month

	Mean individual variation %	Variation of the group %
Peak flow	18.8	3.5
1st min flow	22.1	6.2
2nd min flow	24.6	6.2
5th min flow	22.7	4.8

instead of to its ultimate end. The same reasons hold as for the ischemic exercise hyperemia. Moreover similar conclusions can be made from a study of the ratios of the first min. flow to the corresponding stages of the hyperemia (table IX).

Again an evaluation of the spontaneous variation of the results obtained by the free exercise test (table X) shows how the variation can be reduced to a reasonable order by examining a small group of patients instead of single patients.

Comments

The present analysis of the circulation provides a basis for the use of exercise tests in the estimation of the skeletal muscle blood flow. In addition it corroborates the earlier presented view concerning the distinction between a true improvement of the circulation and an increased flow due to augmented stimulation. This concept may prove to be essential for the understanding of flow physiology and for the correct measurement of the circulation.

Some of the information obtained may also be of importance for the employment of other methods in circulation research such as dye dilution tests or clearance tests. If the method is based, for instance, on assessing the peak flow it should be recognized that unlike the peak flow following ischemic exercise, that following free exercise reflects the flow poorly. On the other hand when the slope of the hyperemic curve or the size of the hyperemia is assessed the free exercise test is then superior.

Furthermore, the present report offers data on two items which are of significance both practically and theoretically. First it must be emphasized that a quantitative debt theory can not be applied to the skeletal muscle blood flow because the flow response to a standard amount of ischemic work was greater in the diseased limbs than in the normal ones. On the other hand a considerable improvement of the circulation through the diseased limbs was followed by an increase of the hyperemia in response to ischemic exercise.

The pathologic hyperemia itself is above all characterized by a loss of flow in the initial period and a subsequent compensatory prolongation. This is an over shooting prolongation so that the size of the hyperemia becomes abnormally great. In understanding this phenomenon the report by Abramson et al. (1) is useful. They examined the hyperemia following anaerobic work in normal subjects and noted that during the initial period of the hyperemia there was an increase both of the flow and of the oxygen extraction. This was followed by a longer period with elevated flow associated with a normal or a decreased oxygen extraction. If these findings hold for the pathologic hyperemia the latter must be prolonged more than expected merely from the initial loss of flow.

the initial loss of flow of the pathologic hyperemia is compensated for by an over-shooting prolongation of its duration. The findings are discussed, and it is concluded that the removal of a possible metabolite is not critically dependent on the magnitude of the post-exercise hyperemia.

The findings of the report provide a basis for a clinical use of exercise tests in assessing the peripheral blood flow. From application of the tests to series of normal limbs and limbs with obliterative arterial disease, their use and significance are illustrated.

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expected when claudication occurs It is reasonable then to consider the effect of pain It is well known that pain can act upon the vasomotor center and thereby bring about a sympathetic vasoconstriction As both skin and muscle are supplied with constrictor nerves, a vasoconstriction in these areas might be the cause of the above-mentioned phenomenon.

Usually the skin flow must be supposed to represent an insignificant share of the post-exercise flow Redisch et al (29) have certainly provided evidence that exercise increases skin and muscle flows by equal amounts in limbs with obliterative disease. In contrast Winsor et al (34) have found blood to be diverted from skin to muscle under the same circumstances. This is largely in line with the report by Coles and Cooper (11) who conclude that the postexercise hyperemia is confined to the tissue deep beneath the skin.

A vasoconstriction is also unlikely to take place in the muscle during work, because such an active tissue has been proved unresponsive to sympathetic stimulation (31) Moreover several authors have found the post-exercise hyperemia unchanged after sympathectomy (8, 20 33)

An obstacle to the venous outflow from the lower limb might affect the hyperemia. Such an obstacle could be caused by the irregular breathing connected with the sensation of pain There may occur a kind of Valsalva s monoeuvre which increases the abdominal pressure and thereby interferes with the venous return from the limb Unfortunately this report can not offer any information on the suggested effect of irregular breathing

At present the remarkable behavior of the circulation in the condition of claudication must remain an unsolved problem

Summary

The blood flow of the calf in response to rhythmic exercise of the calf muscles has been studied in normal limbs and in limbs with intermittent claudication of the calf The blood flow has been measured by means of venous occlusion plethysmography

The typical effects of restricted blood supply on the post-exercise hyperemia are delineated. In short there is an initial loss of flow compensated for by an extended duration of the hyperemia.

Under normal circumstances the flow response is linearly related to the amount of exercise, provided this exceeds a certain small value. For small amounts of ischemic exercise the response is somewhat greater than expected from the slope otherwise found for the responses. The converse holds for the normal response to small amounts of free exercise the flow requirement can probably be satisfied completely by the blood flow during the work.

The pathologic flow response shows an essentially linear relationship to the amount of ischemic and free exercise except for exercise of such an order as to precipitate claudication. The response is then less than expected from the slope of the other responses. The experiments provide no evidence that this phenomenon is caused by vascular spasms.

The fundamental difference between an improvement of the circulation and an increased flow due to an augmentation of the stimulus is delineated

Furthermore, evidence is adduced that a quantitative debt theory cannot be applied to the skeletal muscle circulation Notably in response to ischemic exercise the pathologic post-exercise hyperemia was greater than normal It appears that

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The Peripheral Blood Flow in Intermittent Claudication

VII. The Difference Between the Hyperemias Following Free and Ischemic Exercise and the Effect of the Included Period of Ischemia upon th Latter A Comparison of the Tests for Evaluation of the Blood Flow and of Various Methods for Gauging the Hyperemia. A Note on the Use of Plethysmography in Clinical Studies

By

Leif K. Hillestad

In the concluding paper of the present study the analysis of the blood flow re sponse to exercise is completed. An evaluation is also made of the effect of the circulatory arrest included in the ischemic exercise procedure and it is demonstrated how the hyperemia following ischemic exercise can be corrected for this effect.

Moreover the merits of the various tests in reflecting the actual blood upply are compared and the preferable test for clinical use pointed out.

Another essential problem dealt with is the assessment of the hyperemia produced by these tests. Beside the volumetric method used in the present study it is possible to gauge the hyperemia by means of its duration or of the slope of its declining curve. A comparison of these three method is presented.

Submitted for publication May 24 1963.

Finally some comments and illustrative examples are given on the use of plethysmography in clinical studies.

Material and methods

For these points reference is made to previous papers of this series.

Results

The d fference between the hyperemias following standard period of ischemic and free xercise

As demonstrated earlier the hyperemia following rhythmic exercise with free circulation increased when there was advancing obliterative disease because the blood flow during the exercise became less and less.

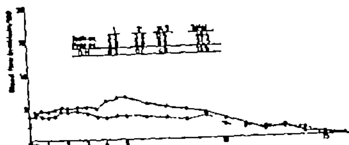


Fig. 2. The hyperemias of the calf following 1 min. of ischemic (●) and 1 min. of free ( ) exercise in limb with complete main artery stenosis.

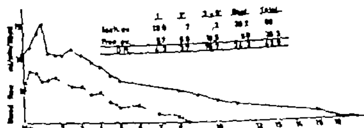


Fig. 3. The hyperemias of the calf following 1 min. of ischemic (●) and 1 min. of free (○) exercise in limb with complete main artery stenosis and well-developed collateral circulation.

Table I. The difference between the hyperemias (excess blood flows) following 1 min. of ischemic and free exercise in 22 normal limbs, 10 limbs with incomplete main artery stenosis and 13 limbs with complete main artery stenosis

	Difference between the excess blood flows (ischemic ex. — free ex.) in ml/min/100 ml					
	1st min	2nd min	3rd–5th min	Rest	Total	Peak
Normal limbs	8.0	5.6	5.5	0.4	14.7	17.8
Incompl. stenosis	3.4	4.6	5.8	0.7	16.5	8.3
Complete stenosis	0.4	0.8	4.0	3.7	8.9	1.1

Table II. The difference between the hyperemias of the calf following 1 min. of ischemic and free exercise in limbs with complete main artery stenosis and varying extents of collateral circulation

Collateral circulation	Difference between the excess blood flow (ischemic ex. — free ex.) in ml/min/100 ml					
	1st min	2nd min	3rd–5th min	Rest	Total	Peak
Excellent	4.3	4.1	6.7	5.3	20.4	6.1
Medium	1.4	2.5	5.6	8.5	18.0	3.0
Poor	0.4	0.8	4.0	3.7	8.9	1.1

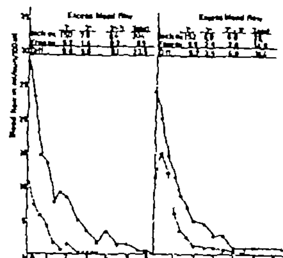


Fig 1 The hyperemia (excess blood flows) of the calf following 1 min of ischemic exercise (●) and 1 min of free exercise (o) in normal limb (left frame) and in a limb with incomplete main artery stenosis (right frame)

Conceivably then the hyperemia produced by free exercise in advancing obliterative disease might progressively approach in size the hyperemia produced by a similar period of exercise with arrested circulation i e. ischemic exercise.

This prediction largely holds true. In passing from the limb with patent arteries over to the limb with incomplete main artery stenosis (fig 1) and further to the limb with a complete main artery stenosis (fig 2) the difference between the respective hyperemias apparently becomes less and less However in this respect the picture of the limbs with a complete main artery stenosis can be influenced considerably by the amount of collateral flow present in each case (fig 3)

A closer analysis reveals that if this difference is to be taken as an index of the blood supply then only the peak flow and the initial parts of the hyperemia are useful (table I) For the later parts of the hyperemia the small differences are maintained in the limbs with complete stenosis, but again these figures may be significantly altered by the varying extents of collateral flow (table II) With excellent collateral flow the figures may approach or be similar to those of the limbs with incomplete stenosis. It can also be observed how the collateral flow prolongs the hyperemia. Even if the difference between the initial parts of the hyperemias remains small, the total difference becomes great. If this difference is to be taken as an index of the actual blood flow it therefore seems that only the initial parts of the hyperemias can be used

For the practical employment of the above index it is useful to ascertain its spontaneous variation. As might be expected on combining the two exercise tests the spontaneous variation is great (table III)

The effect of the period of circulatory arrest included in the ischemic exercise

The ischemic exercise differs from the free exercise in two respects The exercise is made under anaerobic conditions and contains a period of circulatory arrest.

In a normal limb the interrelationship between the hyperemias produced by the same period of ischemic exercise, free exercise and circulatory arrest will vary considerably but a certain general pattern is encountered (fig 4 left frame) The hyperemia produced by the circulatory arrest has a first component above and a second below the line for the blood flow at rest

It is now evident that the initial stage of the hyperemia following ischemic exercise is very similar to the hyperemia following circulatory arrest for the same period This finding is not merely a coincidence The area of the ischemic exercise hyperemia above the line marked *a* in the figure measured in eleven normal limbs, averaged about 1 9 ml/min./100 ml

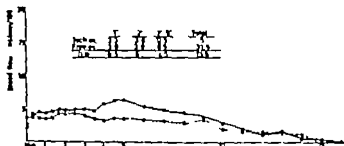


Fig. 2. The hyperemias of the calf following 1 min. of ischemic (●) and 1 min. of free (○) exercise in limb with complete main artery stenosis.

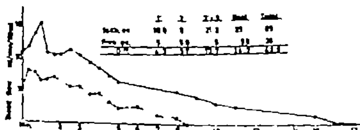


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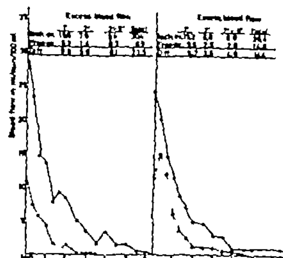


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able V Comparison of the properties of the reactive hyperemia test and the exercise tests in reflecting the ctual blood flow. The hyperemia of the calf following 5 min. of circulatory arrest 1 min. of ischemic and ree exercise as obtained before and after reconstructive surgery in 13 limbs with intermittent claudication lean values are given in the table

	Excess blood flow in ml/min/100 ml					
	1st min	2nd min	3rd–5th min	Rest	Total	Peak
Before op.						
Circulatory arrest	4.3	2.4	1.2	0.0	7.9	6.7
Ischemic exercise	3.6	3.2	10.3	9.2	30.3	9.6
Free exercise	3.2	4.4	6.3	5.5	21.4	8.5
Ischemic ex. — free ex.	0.4	0.8	4.0	3.7	8.9	1.1
After op.						
Circulatory arrest	11.1	2.8	1.0	0.0	14.9	23.1
Ischemic exercise	14.9	8.3	10.8	5.5	39.5	25.3
Free exercise	6.5	3.2	4.2	1.0	14.9	11.3
Ischemic ex. — free ex.	8.4	5.1	6.6	4.5	24.6	14.0

tion of the ischemic exercise hyperemia is made in a limb with a complete main artery stenosis (fig 5) the difference becomes only the half of the normal value. It is reasonable therefore, to suppose that this difference reflects the actual blood supply and also roughly indicates the amount of blood passing through the calf during the exercise.

However these suggestions cannot be maintained because of the effect of the collateral flow As earlier demonstrated, the collateral flow tends to produce unduly prolonged hyperemias, so that the difference becomes even greater than normal. This can be observed by comparing this difference in a series of normal limbs with that in series of ischemic limbs with arying extents of collateral flow (table IV)

In conclusion, it must be stated that the circulatory arrest included in the procedure of ischemi exercise is responsible for only a small part of the hyperemia following exercise with arrested circulation.

Table VI The increase of th 1st min. flow the total hyperemic flow and the peak flow produced by the operation. The calculations are made from the data in table V

	Increase in %		
	1st min.	Total hyperemia	Peak
Circulatory arrest	160	86	245
Ischemic exercise	166	30	164
Free exercise	25	−30	133
Ischemic ex. — free ex.	2,000	173	1173

Comparison between the various tests for evaluating the capacity f the calf blood flow

Such a comparison is best carried out on a series of limbs which have been successfully operated on for major main artery stenosis, because the improvement of the circulation is great enough to provide a clear answer to the problem (table V) At the same time it is not advisable to draw unreserved conclusions from

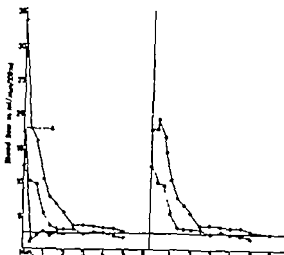


Fig 4 The calf hyperemias of a normal limb in response to 1 min. of ischemic exercise (●) 1 min. of free exercise (o- -o) and 1 min of circulatory arrest (o——o) In the left frame In the right frame the hyperemia in response to ischemic exercise is corrected for the effect of the circulatory arrest



Fig 5 The same experiment as in fig 4 carried out in a limb with complete main artery stenosis.

Table III The spontaneous variation of the difference between the hyperemias following 1 min of ischemic and free exercise in the course of 4 subsequent months in 10 limbs with intermittent claudication, which remained stable during the observation time The limbs were examined once a month

	Mean individual variation (%)	Variation of the group (%)
Peak flow	46.4	12.6
1st min.	78.0	8.2
2nd min.	42.1	4 1
5th min.	54.9	11 7

Table IV The hyperemia of the calf following 1 min of ischemic exercise as corrected for the effect of the included period of circulatory arrest and the hyperemia following 1 min. of free exercise Mean values of the calf blood flow in 11 normal limbs and 12 limbs with intermittent claudication

	Excess blood flow in ml/min/100 ml				
	1st min.	2nd min.	Rest	Total	Peak
Normal limbs					
Ischemic	9.5	5.2	6.2	20.9	13.1
Free	6.8	2.0	0.0	10.2	14.5
Pathol. limbs					
Ischemic	8.1	7.9	11.8	27.8	8.6
Free	7 0	3.8	5.8	16.6	11.0

If the area of the hyperemia following circulatory arrest is measured in the same way for the part above the line for the blood flow at rest the mean is about 2 0 ml/min./100 ml.

In correcting the ischemic exercise hyperemia for the effect of the included period of circulatory arrest, it must first be recognized that the hyperemia of the latter consists of two components, which act conversely The first part, situated above the line for the resting blood flow must be subtracted from the hyperemia produced by the ischemic exercise in the same period. The second part, situated below the line for the blood flow at rest, has similarly to be added to the ischemic exercise hyperemia in the same period. The course of the hyperemic curve is thereby significantly changed (fig 4 right frame) but the hyperemia still differs from that produced by a corresponding amount of free exercise. If the same correc

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Ischemic exercise	5.6	5.2	10.3	9.2	30.3	9.6
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Such a comparison is best carried out on a series of limbs which have been successfully operated on for major main artery stenosis, because the improvement of the circulation is great enough to provide a clear answer to the problem (table V). At the same time it is not advisable to draw unreserved conclusions from

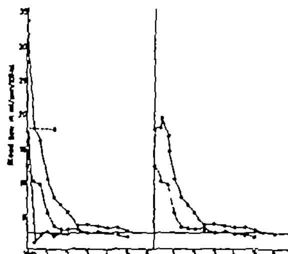


Fig 4 The calf hyperemias of a normal limb in response to 1 min. of ischemic exercise (●) 1 min. of free exercise (o- -o) and 1 min. of circulatory arrest (o——o) in the left frame. In the right frame the hyperemia in response to ischemic exercise is corrected for the effect of the circulatory arrest



Fig 5 The same experiment as in fig 4 carried out in a limb with complete main artery stenosis.

Table III The spontaneous variation of the difference between the hyperemias following 1 min of ischemic and free exercise in the course of 4 subsequent months in 10 limbs with intermittent claudication which remained stable during the observation time The limbs were examined once a month

	Mean individual variation (%)	Variation of the group (%)
Peak flow	46.4	12.6
1st min.	78.0	8..
2nd min.	42.1	4 1
5th min.	54.9	11 7

Table IV The hyperemia of the calf following 1 min of ischemic exercise as corrected for the effect of the included period of circulatory arrest and the hyperemia following 1 min. of free exercise Mean values of the calf blood flow in 11 normal limbs and 12 limbs with intermittent claudication

	Excess blood flow in ml/min/100 ml				
	1st min.	2nd min.	Rest	Total	Peak
Normal limbs					
Ischemic	9.5	5.2	6.2	20.9	13.1
Free	6.8	2.0	0.0	10.2	14.5
Pathol. limbs					
Ischemic	8.1	7.9	11.8	27.8	8 6
Free	7 0	3.8	5.8	16.6	11.0

If the area of the hyperemia following circulatory arrest is measured in the same way for the part above the line for the blood flow at rest the mean is about 2.0 ml/min /100 ml

In correcting the ischemic exercise hyperemia for the effect of the included period of circulatory arrest it must first be recognized that the hyperemia of the latter consists of two components which act conversely The first part situated above the line for the resting blood flow must be subtracted from the hyperemia produced by the ischemic exercise in the same period The second part situated below the line for the blood flow at rest, has similarly to be added to the ischemic exercise hyperemia in the same period. The course of the hyperemic curve is thereby significantly changed (fig 4 right frame) but the hyperemia still differs from that produced by a corresponding amount of free exercise If the same correc

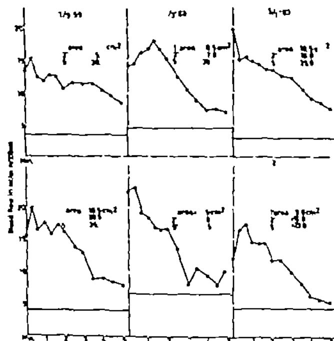


Fig. 8. The hyperemias of the calf in response to 1 min. of ischemic exercise in two different limbs with intermittent claudication (upper and lower frame) examined at identical intervals.

rise of the total hyperemia. In contrast the free exercise test is inferior the increases in the peak flow and the first minute flow being small such that they do not give a true picture of the improvement. On the other hand the free exercise test is the only one that produces a definite decrease of the total hyperemia if there is an improvement of the blood flow

The conclusion from the above analysis is that the reactive hyperemia test based upon the procedure of 5 min of circulatory arrest, is the most suitable test for clinical studies. This conclusion is supported by the outcome of the various tests in limbs with very advanced obliterative disease (fig 6) where the hyperemia following circulatory arrest subsides with in reasonable period of time, whereas the hyperemias following muscular exercise can last for nearly one hour in the same cases. However it should be stated that the conclusions drawn from such clinical studies naturally will gain in certainty and significance if supplemented by the exercise tests.

Comparison of some methods for gauging the hyperemia

Under normal circumstances a hyperemic curve will decline in a roughly exponential manner (fig 7 left frame) so that the curve will become almost a straight line when transferred to a semi-log paper (2) This is, however not always th case for normal hyperemias, especially for those produced by ischemic (1) and free exercise. In pathologic limbs the hyperemic curves are often very irregular and can sometimes be of bizarre shapes (fig 7 right frame) Such curves cannot be straightened out by means of semi-log paper

The hyperemia can be assessed by estimating the volume of the blood contained in it. This volumetric method has

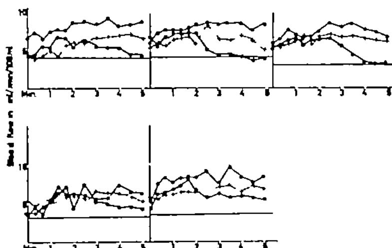


Fig. 6. Hyperemias of the calf in response to 1 min. of ischemic (●) and free (×) exercise and 5 min. of circulatory arrest (o) in a limb with advanced obliterative arterial disease and a poor collateral supply

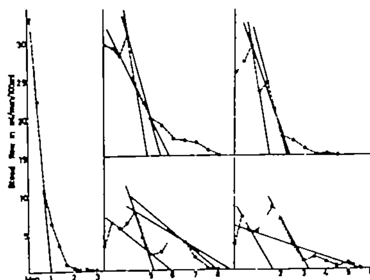


Fig. 7. Hyperemic curve in response to 5 min. of circulatory arrest in a normal limb (o- -o, left frame). Some hyperemic curves with irregular course and many possible slopes (——) as obtained in response to the same procedure in limbs with intermittent claudication (right frame)

such a material for the reason that these limbs are not completely representative in a certain respect. They all have only a poor collateral supply and it has already been pointed out that varying extents of collateral flow can interfere with otherwise regular responses.

In comparing the tests as to their ability to reflect a change of the circulation the difference of the hyperemias following a standard period of ischemic and free exercise is seen to be the best measure (table VI). This method is, however, laborious and entails a great spontaneous variation. In addition the behavior of the total difference of the exercise hyperemias may not be so simple to use in cases with a good collateral flow. As such cases are not subjected to reconstructive surgery this point cannot be evaluated.

The circulatory arrest has the advantage of being easy to carry out, and the subsequent hyperemia passes off relatively quickly. The increase of the peak flow, the first minute flow and the total hyperemia are very satisfactory.

Almost as useful is the ischemic exercise test except for the much smaller

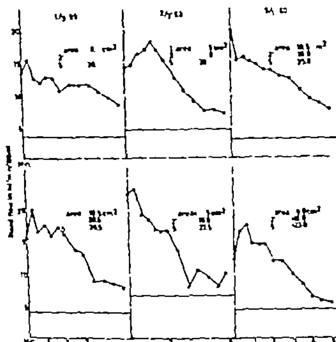


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The hyperemia can be assessed by estimating the volume of the blood contained in it. This volumetric method has

Table VII Comparison between various methods for assessing the hyperemia of the calf in response to 5 min of circulatory arrest. The time at which the hyperemia subsides (duration) the time at which the tangent of the hyperemic curve cuts the line of the resting blood flow (downward slope) and the peak flow Average data from 76 experiments in 48 normal limbs and 84 experiments in 15 limbs with intermittent claudication

	Duration (sec)	Downward slope (sec)	Peak flow (ml/min/100 ml)
Normal limbs			
Mean	140.5	50.2	28.2
S.D	30.7	16.6	7 6
Range	60–240	25–110	13–48
Mean±2.6 S.D	60–221	7– 93	8.4–48
Pathol limbs			
Mean	201.5	120 0	10.3
S.D	78.0	40.0	4.5
Range	70–300	60–270	4–27
Mean±2.6 S.D.	0–404	16–224	0–22

Table VIII Comparison between the duration, the slope and the peak flow in gauging the hyperemia of the calf in response to 1 min. of ischemic exercise Average data from 34 experiments in 22 normal limbs and 77 experiments in 15 limbs with intermittent claudication

	Duration (sec)	Downward slope (sec)	Peak flow (ml/min/100 ml)
Normal limbs			
Mean	254 0	50.2	29.8
S.D	67.5	30 7	6.0
Range	150–400	25–150	18.5–42.0
Mean±2.6 S.D.	79–430	30–130	14.2–45.4
Pathol. limbs			
Mean	424.3	263.0	10.7
S.D	117.2	227 0	5.0
Range	300–720	80–720	6.0–27.0
Mean±2.6 S.D	120–729	0–855	0.0–23.7

Table IX Comparison between the duration, the slope and the peak flow in gauging the hyperemia of the calf in response to 1 min of free exercise From the same material as in table VIII

	Duration (sec)	Downward slope (sec)	Peak flow (ml/min/100 ml)
Normal limbs			
Mean	130.0	68.4	12.0
S.D.	52.4	25.3	3.8
Range	40–240	30–110	4.5–15.5
Mean±2.6 S.D	0–266	3–134	2.1–21.9
Pathol. limbs			
Mean	380.0	274.8	9.2
S.D	102.2	105.3	3.1
Range	210–800	90–800	6.5–13.5
Mean±2.6 S.D.	114–646	1–349	1 1–17.3

been used in the present study However it is also possible to gauge the hyperemia by means of its duration. The time is then taken from the point where it starts to the point where the hyperemic curve attains the line for the blood flow at rest. The third possible method is to draw the slope of the hyperemic curve and use the time at which this slope crosses the line for the resting blood flow as a measure of the hyperemia.

One could also use the angle formed between the slope and the horizontal line as an index, but in practice this method does not work. Especially when applied in limbs with an excellent collateral flow the method can give wrong results. Such limbs usually have hyperemias which are characterized by delayed peak flows and a normal rate of falling off from these peak flows. For this reason a closer analysis of this method has not been conducted

A serious difficulty is encountered in using the slope. An exactly constructed

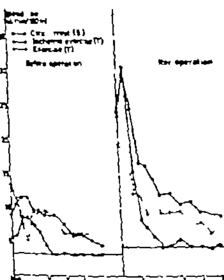


Fig. 9 A typical graph from the follow-up examinations made of the calf blood flow in patients undergoing reconstructive arterial surgery for intermittent claudication.

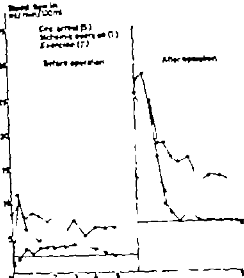


Fig. 10 Graph similar to that in fig. 9

slope would be identical with the regression line through all the declining points of the hyperemic curve. The calculation of such a regression line for each hyperemia would, however, require so much time as to render the procedure impracticable. Consequently the slope has to be drawn in freehand and therefore becomes dependent upon some sort of guess-work. Even an experienced investigator may have trouble in deciding the correct slope. Severe discrepancies may occur between estimations of the actual blood flow made by means of the slope method and those made by means of the volumetric method (fig. 8).

In the present study the slope was drawn according to a predetermined criterion, namely to match the most rapid decline of the hyperemic curve, where the decline was of such a length that it could not be merely a fortuitous departure from the main trend of the curve.

In comparing the three methods for gauging the hyperemia following 5 min. of circulatory arrest (table VII) it is useful to consider the distance between the normal mean plus 2.6 S.D. and the pathologic mean. This distance can be regarded as a measure of the capacity of each method for gauging the hyperemia. It is then observed that the slope is superior to the duration. However since the peak flow in contrast to the other methods shows a greater value for the normal than for the pathologic mean, the figures for the peak flow can be regarded from an opposite point of view. The 2.6 S.D. can be added to the pathologic mean, which then becomes 22 ml. If this figure is compared with the normal mean, the peak flow seems as suitable as the slope for gauging the hyperemia.

It can therefore be concluded that the slope gives the best distinction and that the peak flow comes next to it, while the

Table X The results of a double-blind stud of the effect of anticoagulant treatment of intermittent clau dication due to obliterative arteriosclerosis The group maintained on anticoagulant therapy (phenylindan dione) comprised 14 limbs of 10 patients whose average age was 58.5 years The placebo group comprised 13 limbs of 10 patients, whose average age was 55 8 years The calf blood flow was estimated at rest and following 5 min of circulatory arrest. The subsequent hyperemia is expressed by means of the peak flow and the ratio of the 1st min flow to the remaining hyperemia Mean values are given in the table

	Phenyl indandione group		Placebo group	
	First ex.	Last ex.	First ex.	Last ex.
Calf blood flow at rest (ml/min/100 ml)	3.5	3 4	3.3	3.3
Peak flow (ml/min/100 ml)	14.7	17.3	11.8	14 6
Ratio	3.1	3.8	8.7	11.6
Observation tim (months)	9.0		8.5	

duration obviously is the least useful measure.

A comparison of the three methods in gauging the hyperemia produced by 1 min of ischemic exercise gives an iden tical result (table VIII) An interesting observation is that the normal slope is of the same order for the hyperemias follow ing circulatory arrest and ischemic exer cise. This accords well with the earlier demonstration that the circulatory arrest included in the ischemic exercise is respon sible for the initial part of the hyperemia produced by ischemic exercise

For the hyperemia following 1 min. of free exercise, the outcome of the compari son between the three methods is different (table IX) Again the slope is superior but the duration is not so bad an index as for the other hyperemias. Here, however the peak flow proves quite useless.

Obviously the best measure of the hyperemia is the time from its start until the point where the slope of its decline crosses the level of the resting blood flow However due to the irregular course of the pathologic hyperemias the construc tion of a correct slope will always re main difficult. Indead it seems as good to use the peak flow and carry out an addi tional analysis of the composition of the hyperemia. This does not hold similarly for the hyperemia following free exercise as outlined above.

Examples of the clinical use of plethysmography

As an aid for the surgeon performing reconstructive vascular operations, ple thysmographic examinations serve a use ful purpose (figs. 9 and 10) The result is clearly illustrated and easily understood.

Plethysmography is also practicable in long term clinical studies. The example presented is from a long term study on the effect of anticoagulant treatment in intermittent claudication For several reasons the results of the study were in conclusive. Anticoagulant therapy is sole ly a prophylactic treatment (3) but a majority of the patients included in the double-blind study already had their oc cluding main artery thrombosis. This will most often be the case with patients re ferred to a hospital for intermittent claudication In addition the trial had to be stopped after less than one year of ob servation, because two deaths from coro nary thrombosis occurred in the placebo group

Nevertheless the study gave some useful information. Judged by the reactive hyperemia test (table X) the calf blood flow improved slightly and evenly in both

Table XI. The difference between the peak flow of the hyperemia following 1 min. of ischemic exercise and the peak flow of the hyperemia following 1 min. of free exercise at the monthly examinations during the observation period. Average data are given for the group treated with phenylindanedione and the group treated with placebo. The ratio of the 1st min. flow to the flow during the 2nd, 3rd, 4th and 5th min. of the hyperemia increased for both groups

	Difference between the peak flows (ml/min/100 ml)								
	Months								
	1	2	3	4	5	6	7	8	9
Phenylindanedione group	4.6	5.7	5.1	5.5	6.0	6.2	6.6	7.1	6.9
Placebo group	4.5	4.2	4.1	5.1	5.9	6.4	6.4	6.6	—

groups. A similar result was also obtained by means of the exercise tests (table XI). The mean blood flows showed by all tests a fairly regular course throughout the study. There was nothing to indicate that thrombosis of the collaterals and subsequent formation of new collaterals was a periodically occurring event in either group.

The mean walking tolerance as measured on a tread-mill increased evenly and in correspondence with the blood flow in both groups. As a rule these two factors tallied well in the individual patient (fig. 11), but exceptions were encountered. In some patients the walking distance increased significantly in the presence of a stable blood flow, in others considerable variations of the distance occurred without a similar variation of the blood flow (fig. 12). Several patients came to examination outside the regular intervals because of sudden deteriorations. In no instance could a significant decrease of the blood flow be demonstrated. Usually minor disorders of the locomotor system could explain their symptoms.

The walking tolerance as measured under controlled conditions will be quite a reliable measure of the extent of obliterative disease, but information on the blood flow proper is always essential in the study of ischemic disease. For this purpose plethysmography is at present to be regarded as the method of choice.

Comments

From a theoretical point of view it is reasonable that there should occur a diminution of the difference between the hyperemias produced by similar amounts of ischemic and free exercise with advancing obliterative disease. However this prediction is wrong, especially in cases with a good collateral flow. The hyperemias then become so prolonged that the total difference between them may exceed the normal value, but still the difference remains small in the initial period of the post-exercise flows. This initial difference can therefore be used as an index of the actual blood supply and it also proves to be an extremely sensitive index. The experiments indicate that the collateral flow is poorly regulated with respect to precise relationships between work and flow. It appears that once the collateral flow has been put into action, it persists for a longer period than required.

As demonstrated, the circulatory arrest included in the ischemic exercise seems to be responsible for the initial period

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	Months								
	1	2	3	4	5	6	7	8	9
Phenylindandione group	4.6	5.7	5.1	5.5	6.0	6.2	6.0	7.1	6.9
Placebo group	4.5	4.2	4.1	5.1	5.9	6.4	6.4	6.8	—

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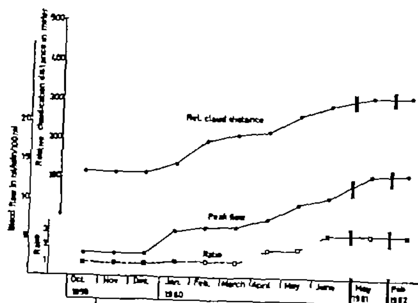


Fig 11 Long-term study of the walking tolerance and the calf blood flow in a patient with intermittent claudication treated with phenylindandione. The peak flow and the ratio are taken from the hyperemia of the calf following 5 min. of circulatory arrest.

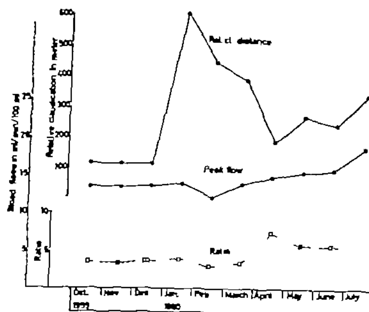


Fig 12 From the same long-term study as fig 11 Exceptionally great variation of the walking tolerance in a period with stable blood flow

of the hyperemia produced by the latter If this hyperemia could be corrected not only for the effect of the circulatory arrest, but also for the effect of the anaerobic versus the aerobic work, the blood flow during the free exercise could possibly be calculated. This blood flow would then approximate to the difference between the ischemic exercise hyperemia, corrected for the effect of the circulatory arrest and the anaerobic work, and the free exercise hyperemia. Unfortunately a method for estimation of the blood flow during exercise does not exist (4)

It is useful to know that the simple reactive hyperemia test reflects the capacity of the peripheral blood flow as well as the more laborious exercise tests. The test employing the difference between the initial parts of the exercise hyperemias is

much more sensitive but usually the same kind of information will be obtained by means of the simple reactive hyperemia test. This indicates that the test used probably is of less importance than the position of the plethysmograph during the examination. When information on the skin blood flow is wanted the apparatus is placed at a part of the limb consisting mainly of skin, and when information is wanted on the muscle blood flow the plethysmograph has to be placed at a part consisting mainly of muscle. In both places the reactive hyperemia test can be used for evaluating the capacity of the blood supply This simplifies the procedure considerably

In gauging the hyperemia the slope of the hyperemic curve proved to be the best index. However it is a disadvantage that this slope cannot be exactly drawn without so much work as to make the method impracticable. It may be feasible to draw the slope freehand, but the doorway is at the same time opened for the bias of the investigator The volumetric method leaves no room for doubt as to what is correct or not. In the analysis presented, only the peak flow was evaluated together with the methods based on slope and duration. As demonstrated earlier the volumetric method gives not only the peak flow but also an additional analysis of the various parts of the hyperemia. The value of the volumetric method is therefore greater than was apparent from the comparative analysis of the methods for gauging the hyperemia. However as the slope method is easy to apply it may be used as an adjunct to the volumetric method.

The long-term study of the patients with intermittent claudication has shown how plethysmography can be applied. It also showed that on the average the blood flow in intermittent claudication is apt to increase a little with time. This accords with the present concept of intermittent claudication as a benign disease and not a harbinger of gangrene. These patients are threatened by fatal catastrophes from other organ systems.

As is also demonstrated it is not advisable to attribute every change of the walking tolerance to a change of the blood flow It should therefore be emphasized that conclusions as to the actual blood flow should not be drawn without a study of this subject.

Summary

The difference between the initial parts of the hyperemias produced by identical amounts of ischemic and free exercise is shown to be a sensitive index of the peripheral blood flow The total difference between the hyperemias is useless in this respect, because the total difference between the prolonged pathologic hyperemias often exceeds the normal value.

It is demonstrated that the circulatory arrest included in the ischemic exercise is responsible for the initial part of the hyperemia produced by the latter

A comparison has been made between the reactive hyperemia test and the exercise tests for ability to reflect the blood supply to the calf. It is concluded that the laborious exercise tests do not offer any advantages over the reactive hyperemia test, which is to be preferred also on account of its simplicity

In gauging the hyperemia the slope of the hyperemic curve proved to be best all-round index. For the hyperemias produced by circulatory arrest and ischemic exercise the peak flow was also useful, whereas the duration was a poor measure. For the hyperemia produced by free

exercise the duration seemed to be a good index, while the peak flow was unsatisfactory. These results are discussed and it is concluded that the estimation of the peak flow combined with a volumetric analysis of the hyperemia is still the preferable method.

Some examples are presented on the use of plethysmography in clinical studies of ischemic disease of the lower extremities. The walking tolerance generally corresponds with the actual blood flow, but exceptions are encountered. It is therefore not advisable to draw conclusions as to the blood flow from examinations of the walking tolerance.

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Seromucoid Fractions, Serum Proteins and Lipids in Diabetes Mellitus

By

A. Johnson, J Goa, S E. Fagerberg and B. Hood

A number of studies, using the periodic acid-Schiff staining method as well as Rinehart modification of Hale's colloidal iron staining method (19) have made it clear that diabetic angiopathy may be definitely separated from the atherosclerotic vascular disease (17 13)

Periodic acid-Schiff staining deposits closely similar to those in the kidney and retina have now been found in the small vessels of peripheral nerves (9) of the skin (3) of the stomach (2) of the joint capsules (1) and in the peripheral vessels and the vasa vasorum (13)

The observation of deposits with these staining characteristics has created an interest in the protein-bound carbohydrates of plasma. A number of different parameters have been measured with a variety of techniques The protein bound carbohydrat levels have usually been found to be raised in diabetics as compared with normals, more so in diabetes with vascular complications and especially when renal failure was present.

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A determination of total protein-bound glucosamine, galactosamine etc. will naturally tell just as little as for instance the determination of the individual lipids in a fat solvent extraction of plasma of the molecular species involved and their individual concentrations. Also the simple grouping of the diabetics in the series investigated into uncomplicated and complicated does not take into account that the rate of development of the vascular complication should be correlated with the concentrations of substances suspected to be of importance. In other words a case placed in the group without observable complications at the duration of 5 years may at 10 years of known duration be severely complicated and eventually more so than a number of the cases originally belonging to the group with complications.

It was thus the primary aim of this work to study the individual seromucoid fractions according to the technique of Goa (12) and correlate these fractions

with other serum protein and lipid parameters. We also intended to concentrate on two diametrically opposite groups — one which develops marked angiopathy during a short known duration and the other with no or insignificant angiopathy after a long known duration. However although a large population of diabetics was screened for this purpose, it was impossible to create large enough groups. Cases with long duration of diabetes and insignificant complications were especially difficult to find.

Material

A number of the original cases were dropped from the study as we failed to achieve a state of well regulated carbohydrate metabolism.

The material comprises 70 diabetics with onset before the age of 40 years, 32 women and 38 men. Twenty-six of these were inpatients usually admitted for reasons of severe complications or lack of control or both. In these patients sampling was not done until control had been achieved for more than one week. In a few cases the serum levels were followed from coma or precoma until control had been achieved. These data have not been included in the diagrams and tables representing the main findings. Patients with advanced renal failure, i.e. those with a serum creatine higher than 2.5 mg % (normal values with the present method 0.8—1.4 mg) have also been eliminated as have those with clearcut nephrotic features.

GRADING OF CLINICAL SIGNS OF ANGIOPATHY

Eyegrounds were investigated by an expert ophthalmologist.

Kidneys were assessed by serum creatinine measurements and quantitative urine protein measurements. If urinary sediments showed pyuria or if there was a suspicious history of pyelonephritis the patients were generally submitted to intravenous pyelography, pitressin tannate concentration test and micturition cystography. Renal impairment and proteinurea were only interpreted as signs of angiopathy in the absence of signs of pyelonephritis.

NEUROPATHY

In this investigation both loss of tendon reflexes and definite impairment of the vibratory sense have been required for this diagnosis.

The grading system below has been used.

Retinopathy

Microaneurysm	1
Microaneurysms + haemorrhages and/or exudates	2
Proliferating retinopathy	3

Nephropathy

Constant proteinuria with no other explanation	1
Proteinuria + signs of renal impairment	3

Neuropathy

Definite signs	1

This system would give 7 points for the patient with the most severe clinical manifestations of angiopathy.

In the tables and figures we have used the following symbols.

○ = No clinically observable complications.
◔ = Complications 1 or 2 points.
◑ = Complications 3 points.
◕ = Complications 4 or more points.

Methods

Seromucoid and serum protein determinations were made on sera stored in the frozen state. This did not influence the measurements. Cholesterol was determined according to the method of Cramér and Isaksson (8) and triglyceride to the method of Carlson and Wadstrom (7) on a fresh serum sample. The urine glucose should not exceed 20 g and usually did not exceed 10 g during the 24 hrs preceding the sampling. The fasting serum glucose as practically in all patients below 150 mg on the morning when the blood was drawn.

a) *Total protein* was determined by means of the biuret reaction. A calibration curve was made up by means of micro Kjeldahl analyses and conversion factor for protein of 6.25. Determinations on 30 healthy persons of both sexes and age of 20 to 4 years showed a mean value of 7.0 and a *standard deviation* of

Table I Electrophoretic serum protein fractions in relation to duration and grade of complication

Mean value ± S.D.

Complication grade	Average duration	No.	Total protein	Albumin	α_1	α_2	β	γ
O Duration < 5 years	2.1	10	6.37 ± 0.80	3.05 ± 0.38	0.38 ± 0.06	0.68 ± 0.09	0.93 ± 0.14	1.35 ± 0.35
O Duration > 5 years	10.2	11	7.15 ± 0.68	3.61 ± 0.44	0.34 ± 0.08	0.74 ± 0.14	0.90 ± 0.13	1.58 ± 0.73
◔	19.1	22	6.91 ± 0.42	3.47 ± 0.39	0.35 ± 0.05	0.71 ± 0.35	0.93 ± 0.17	1.43 ± 0.47
◑	18.1	13	6.74 ± 0.70	3.40 ± 0.70	0.38 ± 0.02	0.70 ± 0.37	0.93 ± 0.45	1.28 ± 0.22
◕	16.9	8	6.99 ± 0.35	3.41 ± 0.31	0.38 ± 0.02	0.74 ± 0.09	0.97 ± 0.04	1.41 ± 0.32
Average of the whole material	15.0	64	6.84 ± 0.63	3.41 ± 0.47	0.36 ± 0.02	0.71 ± 0.04	0.93 ± 0.15	1.41 ± 0.28
	Normal values g %		7.0 ± 0.4	4.2 ± 0.7	0.3 ± 0.1	0.5 ± 0.2	0.8 ± 0.3	1.3 ± 0.4

± 0.4 which is in excellent agreement with the values found by Spak (20).

b) *Paper electrophoresis* of serum was carried out in "Elphor chambers" (Bender & Hobein, München) with barbital buffer of pH 8.6, ionic strength 0.10 and 90 V for 16 hours. The paper used was Schleicher & Schüll No. 2043. The papers were stained with amido black B and read in Spinco Analytrol. A correction factor of 1.15 was used for the globulins. Normal values were made up from the same 30 persons as mentioned above. The values represent mean ± s. d. rounded to the nearest tenth of gram. They are given in g/100 ml.

Alb. 3.5—4.9 g/100 ml

α_1 0.2—0.4

α_2 0.3—0.7

β 0.6—1.1

γ 0.9—1.6

c) *Hexosamine* was determined by means of the Elson and Morgan reaction as described by Blix (5).

In 35 blood donors the mean value was found to be 91 mg/100 ml and the standard deviation ± 10 mg/100 ml (11).

d) *Determination of total seromucoids* was determined mainly according to Winzler et al. (21).

Determination of the seromucoid subfractions was carried out according to Goa (12). Four fractions were obtained and called SM I, II, III and IV where I is the fastest moving one.

Procedure

An amount of 5 ml serum was diluted with 5 ml 0.9 % sodium chloride, 10 ml 0.8 M perchloric acid were added and mixed with a glass rod. Filtration was carried out immediately.

For determination of the total amount of seromucoids, 4 ml of the clear filtrate were pipetted and the seromucoids precipitated by addition of 1 ml phosphotungstic acid solution (2 % in 2 N hydrochloric acid) and centrifuged. The supernatant solution was decanted, the tube inverted on to filter paper and the precipitate dissolved in 4 ml of biuret reagent (1 part Benedict copper sulphate solution for glucose mixed with 20 parts 3 %

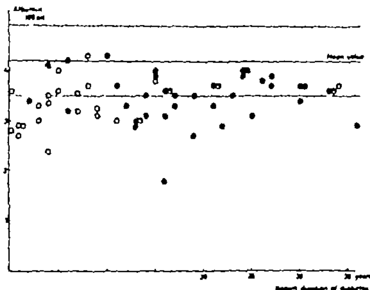


Fig 1 Albumin levels i relation to known duration and grade of complication (see explanation of symbols under material) In all figures mean value stands for normals.

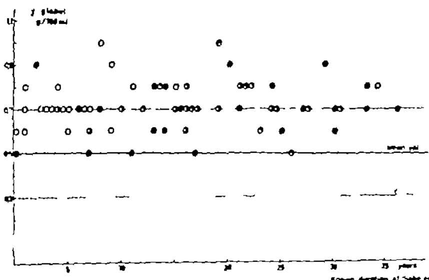


Fig 2. -globulin levels in relation to known duration and grade of complication (see explanation of symbols under material)

sodium hydroxide) The optical density was read after 15 min in a Beckman B (or other suitable photometers) at 330 mμ in 10 mm cells. The reading multiplied by the factor 400 gives the seromucoids in mg/100 ml serum. The rest of the perchloric acid filtrate was precipitated by addition of 1 ml phosphotungstic acid solution After centrifugation the precipitate was dissolved by the addition of 0 1 M sodium hydroxide to pH 6—7 To this solution was added about three times its volume of 95 ethanol and centrifuged. After decanting the precipitate was dried for about 1 min. in a stream of air and finally dissolved in a small volume of distilled water

For normal sera about 30 μl were used, and for sera with higher levels of seromucoids, up to 200 μl were used. Of this solution amounts of 20—40 μl were applied on the paper electrophoresis strips. Electrophoresis was carried out in an apparatus built mainly after the principles given by Grassman and Hannig (14) The buffer was that given by Aronsson and Grönwall (4) but adjusted to pH 8 4

The voltage was 5.5 V/cm, electrophoresis time 18 hours and the paper used was Schleicher and Schüll No. 2043 a. After electrophoresis the paper strips were dried at 100° C, stained with amido-black B and read in a

Spinco Analytrol apparatus. More accurate determinations were obtained by eluting the d[illegible] for 2 hours in 0.05 M sodium hydroxide followed by photometry [illegible]t 620 mμ.

Normal values were determined from the same 30 sera as for total protein and paper electrophoresis. There was no significant difference between the two series.

Total seromucoid 79 [illegible] ± 9, fraction I 8.6, [illegible] ± 2.3 fraction II 36.6, [illegible] ± 6, fraction III 24.8, [illegible] ± 4.6 and fraction IV 9.7 [illegible] ± 2.3.

The values represent mean and standard deviation given in mg/100 ml.

Results

Serum proteins

As seen in table I the figures for total protein, α β and γ globulins are well within normal limits although the a[illegible]erage α_1 [illegible]alues seem somewhat high.

Average albumin levels were close to the lower level of the normal range in all the groups. Most striking are the low levels in the group without complications with duration less than 5 years (fig 1)

A closer study of the individual cases, a number of which had albumin levels between [illegible] and 5 g per cent revealed that most of these cases were either newly discovered or had been admitted because of severe lack of control W[illegible] have tentatively assumed that even if the blood and urine sugar levels had been corrected it is possible in these cases that long-standing metabolic derangement w[illegible]th negative protein balance might not ha[illegible]e been fully corrected [illegible]t the time of sampling

The α levels averaged around 0 7 g per cent (table I and fig 2) In other words [illegible]erage levels were at the upper level of the normal range and no values were below the average for normals. This feature was independent of the known duration of diabetes and seemed to have nothing to do with lack of control, intercurrent disease or any other such features. Johnsson (15) in 15 of 30 patients with diabetes of long duration and insignificant late manifestations found elevated α_2-globulin.

Table II Cholesterol and triglyceride levels in relation to duration and grade of complication

Mean value ± S D.

Complication grade	Cholesterol mg/100 ml	No.	Glyceride-glycerol mM 1/l	No.
[illegible] Duration < 5 years	224 ± 47.8	11	1 18 ± 0.68	11
[illegible] Duration > 5 years	242 ± 49.1	12	1 17 ± 0.37	12
[illegible]	243 ± 47[illegible]	22	1 19 ± 0.44	22
[illegible]	290 ± 81 4	14	1.22 ± 0.70	13
[illegible]	293 ± 63.8	9	1.85 ± 0.76	9
Average of the whole material	256 ± 63.0	68	1.28 ± 0.6	67
Normal values	230 ± 50		1.25 ± 0.35	

Cholesterol and triglyceride measurements

As seen in table II [illegible]verage levels of cholesterol were high only in the two most complicated groups and serum triglyceride only in the group with the most advanced complications.

There was some tendency for the serum triglycerid[illegible] levels to vary inversely with the serum albumin levels, although cases with outspoken nephrotic features had been eliminated. Two cases were borderline in this respect and are included. These two had urine protein output between 3—8 g/24 hrs and [illegible]

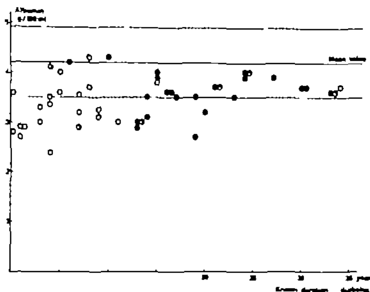


Fig 1 Albumin levels in relation t known duration and grade of complication (see explanation of symbols under material) In all figures mean value stands for normals.

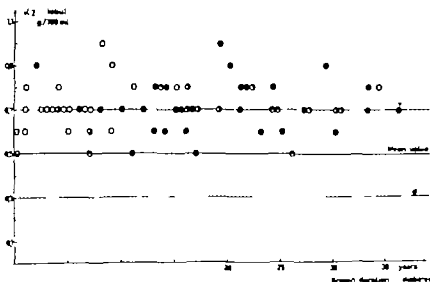


Fig 2. α_2-globulin levels in relation to known duration and grade of complication (see explanation of symbols under material)

sodium hydroxide) The optical density was read after 15 min. in a Beckman B (or other suitable photometers) at 330 mμ in 10 mm cells. The reading multiplied by the factor 400 gives the seromucoids in mg/100 ml serum. The rest of the perchloric acid filtrate was precipitated by addition of 1 ml phosphotungstic acid solution. After centrifugation the precip tate was dissolved by the addition of 0 1 M sodium hydroxide to pH 6—7 To this solution was added about three times its volume of 95 % ethanol and centrifuged. After decanting, the precipitate was dried for about 1 min. in a stream of air and finally dissolved in a small volume of distilled water For normal sera about 50 μl were used, and for sera with higher levels of seromucoids, up to 200 μl were used. Of this solution amounts of 20—40 μl were applied on the paper electrophoresis strips. Electrophoresis was carried out in an apparatus built mainly after the principles given by Grassman and Hannig (14) The buffer was that given by Aronsson and Gron all (4) but adjusted to pH 8.4

The voltage was 3.5 V/cm, electrophoresis time 18 hours and the paper used was Schleicher and Schull No. 2043 a. After electrophoresis the paper strips were dried at 100° C, stained with amido-black B and read in a

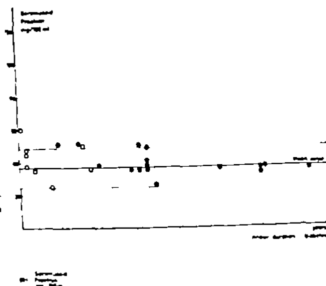


Fig 3 Seromucoid II in relation to known duration and grade of complication (see explanation of symbols under material)

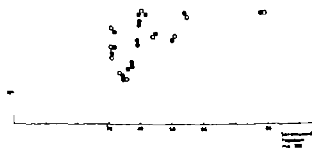


Fig 4 Seromucoid II versus seromucoid III.

values show wider spread. SM I (table III and fig 5) shows the most interesting changes. Approximately two thirds of the observations fall below the mean normal level -2σ and not a single determination reaches the mean normal value. There is a moderate spread of values and the low levels seem independent of the duration and degree of complication. The large number of individuals with no clinically observable complications with short known duration and with low levels of this seromucoid fraction is clearly seen in fig 5 (left lower part)

When the figures for this fraction are plotted against serum albumin levels no consistent pattern is seen (not included in this paper) Extremely low levels of seromucoid I may co-exist with normal levels of albumin and low levels of albumin with moderately depressed seromucoid I

Uremia did not seem to influence the levels of SM I to any marked degree. One patient had a total seromucoid of 92 mg%

Table III Total seromucoids hexosamine and individual seromucoid fractions in relation to duration and grade of complication

Mean value mg/100 ml ± S D

Complication grade	Average duration	No	Total seromucoid	Hexosamine	Seromucoid fractions I	II	III	IV
○ Duration < 5 years	2.1	11	91.2 ± 20.7	86.8 ± 10.8	3.1 ± 1.8	45.2 ± 13.5	32.8 ± 10.0	10.6 ± 4.7
○ Duration > 5 years	10.2	12	82.5 ± 27.9	81.2 ± 25.2	2.9 ± 1.5	42.1 ± 17.8	28.0 ± 11.4	10.9 ± 6.0
◔	19.1	23	85.3 ± 24.7	84.7 ± 8.2	3.4 ± 1.9	45.0 ± 14.3	29.9 ± 11.4	10.0 ± 4.5
◑	18.9	14	87.2 ± 26.7	86.9 ± 12.6	3.2 ± 1.9	44.9 ± 15.2	28.5 ± 11.0	9.8 ± 4.8
◕	16.9	10	106.9 ± 31.2	88.5 ± 12.5	3.1 ± 2.0	55.8 ± 14.5	30.2 ± 10.2	11.2 ± 7.2
Average of whole material	14.5	70	89.3 ± 25.8	85.2 ± 14.1	3.2 ± 1.8	45.3 ± 9.2	29.8 ± 10.4	10.3 ± 5.2
Normal values			78.6 ± 9.0	91 ± 10	8.6 ± 2.5	36.6 ± 6.0	24.8 ± 4.6	9.7 ± 2.3

levels of 3 and 1 8 g respectively In both these cases serum albumin levels were considerably raised in the course of several months on a high protein diet without any marked change occurring in the triglyceride levels. Outside the group with the gravest complications there were only two cases, who exhibited raised triglyceride levels. However this does not necessarily reflect the average triglyceride levels occurring throughout these patients lives as diabetics, since consistent and determined efforts had been made to bring them under as good control as possible before the sampling was made.

Total seromucoid and seromucoid fractions

Total seromucoids show a distinct elevation in the group with most advanced complications (table III) Average value in this group is more than the average normal value + 3 σ In two uremic cases of diabetes who have not been included in the present study values of 276 and 154 mg per cent were found respectively

Of the individual seromucoid fractions SM II (fig 3) which seems identical with orosomucoid (10) and SM III both show some tendency to elevation in all the different groups of diabetics The average level for SM II also seems particularly elevated in the group with the most advanced complications. SM II and SM III show a tendency to vary in a parallel manner as shown in fig 4 SM IV shows in all groups insignificantly higher average values than the normal mean, but the

Table IV Seromucoid levels in coma as compared with a well regulated state

Case no.	Clinical data	Total seromucoid mg/100 ml	Seromucoid fractions			
			I	II	III	IV
1	Diabetic coma	98	3	39	38	18
	3 weeks later — blood and urine sugar levels well controlled	109	3	43	47	16
2	Diabetic coma	89	4	32	38	15
	2 months later — blood and urine sugar levels well controlled	84	2	45	32	5
3	Diabetic coma	128	1	85	27	15
	1 week later — blood and urine sugar levels well controlled	109	3	60	35	11

Summary

1 In 70 diabetic subjects with onset before the age of 40 years clinically manifest complications have been graded, correlated with known length of duration, serum levels of proteins electrophoretically separated seromucoid fractions, cholesterol and triglyceride.

The above serum parameters have been studied when the patient's glucose metabolism has been brought under as good control as is practically possible.

3 Average serum albumin levels were low and average globulin levels were high in all the subgroups of diabetics

4 Total seromucoids were definitely elevated only in the group with the most advanced complications.

5 Of the four individual seromucoid fractions, one, seromucoid IV did not differ from the average value in normals.

6. The two largest fractions, seromucoid II and III were elevated in all diabetic subgroups, fraction II more so in the group with the most advanced complications

7 The most striking finding was the marked depression of the serum level of the newly described seromucoid fraction I which was present independently of duration and severity of complications.

This feature of a low seromucoid I was also found in young diabetics with short known duration, without clinically manifest complications and with well controlled glucose metabolism.

This fraction did not correlate with the serum albumin levels.

8. The average level of cholesterol was only raised in the most complicated group and average triglyceride level only in the one with the most complications. Outside of the most advanced group high triglyceride levels only occurred in two subjects. However it is stressed, that these values were obtained when the diabetics had been brought to a state of control which they usually do not achieve in general life.

9 The marked alterations in the seromucoid and serum protein fractions

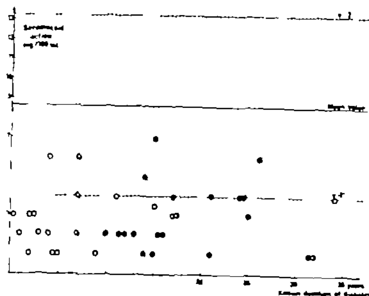


Fig 5 Seromucoid I in relation to known duration and grade of complication (see explanation of symbols under material)

with 1 mg % in this fraction 1/2 year later with increasing uremia he had a total seromucoid of 276 mg % and SM I was 5 mg % Another uremic patient had a total seromucoid figure of 154 mg % with SM I of 2 mg %

Three patients were studied in diabetic coma and also after their carbohydrate metabolism had been corrected (table IV) The levels of SM I and to some extent the other seromucoid fractions seem comparatively stable even in the presence of drastic alterations in carbohydrate metabolism.

Discussion

The pattern of seromucoid and serum protein pattern described in this paper in diabetes can be summarized as follows.

There is a tendency to low albumin and high α globulin levels, high levels of seromucoid II and III and a very low level of seromucoid I This pattern seems by no means specific for diabetes. Goa (12) already illustrated similar changes in hepatitis leukemia and chronic pancreatitis As regards the most striking change, the low levels of seromucoid fraction I two further cases of chronic leukemia and three cases of severe infectious hepatitis showed levels of this fraction of 2 2 2 3 and 3 mg % respectively

The elevated seromucoid levels earlier described may well correspond to the elevations we have observed in the two fractions SM II and III representing the bulk of the total seromucoid

The most striking observation of the present study is that young diabetics in apparent excellent condition with no complications observable on penetrating clinical examination and with well regulated diabetes share this marked alteration of plasma proteins and seromucoids with such disorders as leukemia hepatitis and chronic pancreatitis and probably with a number of other disorders.

The common mechanism behind these alterations should be studied more closely

In this condition the statement of Meier (16) that spontaneous diabetes in the Chinese golden hamster may be presaged by an elevated α globulin level seems of more than passing interest.

Table IV Seromucoid levels in coma as compared with well regulated state

Case no	Clinical data	Total seromucoid mg/100 ml	Seromucoid fractions			
			I	II	III	IV
1	Diabetic coma	98	3	39	38	18
	3 weeks later — blood and urine sugar levels well controlled	109	3	43	47	16
2	Diabetic coma	89	4	32	38	15
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3	Diabetic coma	128	1	85	27	15
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Summary

1 In 70 diabetic subjects with onset before the age of 40 years clinically manifest complications have been graded correlated with known length of duration, serum levels of proteins, electrophoretically separated seromucoid fractions, cholesterol and triglyceride.

2 The above serum parameters have been studied when the patient glucose metabolism has been brought under as good control as is practically possible.

3 Average serum albumin levels were low and average α globulin levels were high in all the subgroups of diabetics.

4 Total seromucoids were definitely elevated only in the group with the most advanced complications.

5 Of the four individual seromucoid fractions, one, seromucoid IV did not differ from the average value in normals.

6 The two largest fractions, seromucoid II and III, were elevated in all diabetic subgroups, fraction II more so in the group with the most advanced complications.

7 The most striking finding was the marked depression of the serum level of the newly described seromucoid fraction I which was present independently of duration and severity of complications

This feature of a low seromucoid I was also found in young diabetics with short known duration without clinically manifest complications and with well controlled glucoses metabolism.

This fraction did not correlate with the serum albumin levels.

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9 The marked alterations in the seromucoid and serum protein fractions

in patients with diabetes seem to be shared with a number of diseases, notably leukemia and severe hepatitis. The occurrence in the young uncomplicated wellcontrolled patient with a short known duration immediately raises the question as to whether they occur before the diabetes becomes clinically manifest. This question is pursued.

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Motor Disturbances in Diabetes Mellitus

A Clinical Study Using Electromyography and Nerve Conduction Velocity Determination

By

S. E. Fagerberg, I. Petersén, G. Steg and L. Wilhelmsen

The intention of this examination has been to study the occurrence of motor disturbances in diabetic neuropathy since, according to earlier studies, such disturbances are surprisingly unusual compared with sensory disturbances. In material presented in 1959 by Fagerberg (2) on diabetic neuropathy consisting of 224 patients where the intraneural vessels were studied, motor disturbances could be shown only in a few cases. Generally speaking it can be said that the same experience dominates in the literature. During the past year some American authors (1, 3, 6, 8, 9) have adopted the same doubtful attitude as our own.

Material and methods

The investigation was performed on a series of 128 consecutive diabetics. To make allowance for any influence on nervous function of unspecific arteriosclerosis and other age changes, the patients were classified according to age in groups of over 60, 40—60 and under 40 years. These groups were in turn divided into patients with and without clinical signs of neuropathy. Eight patients with only EMG signs are included in the group with neuropathy (table I).

Clinical examinations

Each patient was subjected to the following examinations: routine medical examination, neurological examination, fundoscopy (by ophthalmologist), renal function studies, oscillometry and X-ray examination to detect any intravascular calcifications in the legs.

An index of the degree of diabetic angiopathy in the eyes, kidneys and legs was obtained by using point scale (table II) for rating the various manifestations.

Electromyography (EMG)

Electromyograms were recorded from M. interosseus I dorsalis manus, M. tibialis anterior and M. extensor digitorum brevis, using a Disa EMG machine (time constant of one second). The norms for abnormality of an EMG were the conventional ones, i.e. the presence of so-called denervation potentials and of reduced activity in response to maximal voluntary contraction.

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in patients with diabetes seem to be shared with a number of diseases, notably leukemia and severe hepatitis. The occurrence in the young uncomplicated wellcontrolled patient with a short known duration, immediately raises the question as to whether they occur before the diabetes becomes clinically manifest. This question is pursued.

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Motor Disturbances in Diabetes Mellitus

A Clinical Study Using Electromyography and Nerve Conduction Velocity Determination

By

S.-E. Fagerberg, I. Petersén, C. Steg and L. Wilhelmsen

The intention of this examination has been to study the occurrence of motor disturbances in diabetic *neuropathy* since, according to earlier studies, such disturbances are surprisingly unusual compared with sensory disturbances. In material presented in 1959 by Fagerberg (7) on diabetic neuropathy consisting of 224 patients where the intraneural vessels were studied, motor disturbances could be shown only in a few cases. Generally speaking, it can be said that the same experience dominates in the literature. During the past year some American authors (1, 3, 6, 8, 9) have adopted the same doubtful attitude as our own.

Material and methods

The investigation was performed on a series of 178 consecutive diabetics. To make allowance for any influence on nervous function of unspecific arteriosclerosis and other age changes, the patients were classified according to age in groups of over 60, 40—60 and under 40 years. These groups were in turn divided into patients with and without clinical signs of neuropathy. Eight patients with only EMG signs are included in the group with neuropathy (table I).

Clinical examinations

Each patient was subjected to the following examinations: routine medical examination, neurological examination, fundoscopy (by ophthalmologist), renal function studies, oscillometry and X-ray examination to detect any intravascular calcifications in the legs.

An index of the degree of diabetic angiopathy in the eyes, kidneys and legs was obtained by using a point scale (table II) for rating the various manifestations.

Electromyography (*EMG*)

Electromyograms were recorded from M. interosseus I dorsalis manus, M. tibialis anterior and M. extensor digitorum brevis, using a Disa EMG machine, time constant of one second. The norms for abnormality of an EMG were the conventional ones, i.e. the presence of so-called denervation potentials and of reduced activity in response to maximal voluntary contraction.

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Table I. Diabetics with and without clinical neuropathy classified according to age

Age group (yrs)	With neuropathy	Without neuropathy	Total
> 60	38	15	53
40–60	28	23	51
< 40	10	14	24
Total	76	52	128

Table II. Point scale for rating manifestations of diabetic angiopathy

	Points
Microaneurysms	1
Microaneurysms with bleeding or effusion	2
Proliferative changes	3
Proteinuria not due to something other than diabetes	1
Renal insufficiency	3
Signs of angiopathy in legs	1

Motor nerve conduction velocity

Altogether 162 conduction velocity determinations were made in motor fibres of the ulnar and deep peroneal nerves of 50 diabetics — 30 with and 20 without clinical signs of neuropathy — and 34 non-diabetic controls. Among these 84 subjects 33 were over 60, 28 from 40 to 60 and 23 under 40 years old.

The method was essentially that of Helmholz (1852), except for electromyographic rather than mechanical recording, and has recently been used for conduction velocity measurements in diabetics (6, 8, 9). The ulnar nerve was stimulated by skin electrodes at the elbow and at the wrist. The action potentials of the hypothenar muscles were recorded by electrodes attached to the skin over the muscles. Similarly, the deep peroneal nerve was stimulated by one electrode at the proxi- [illegible] from M. extensor digitorum brevis. The conduction time was derived by superimposing the action potentials recorded in response to stimulation and measuring the distance in milliseconds between the two shock artefacts. The conduction velocity was calculated by dividing the distance in millimetres between the stimulating electrodes by the conduction time in milliseconds.

Results

Electromyography

As many as 55 of the 76 patients with diabetic neuropathy exhibited EMG evidence of motor involvement. This is equivalent to 72 per cent and of the entire series of 128 diabetics it is 43 per cent. The abnormal EMG was the only sign of motor defects in 8 patients without sensory disturbances, and without the EMG investigation they would have been classified as diabetics without neuropathy. The incidences of sensory and motor defects, alone and combined, found in the three age groups of patients with diabetic neuropathy are shown in table III.

Mean durations of diabetes in years for 55 patients with motor disturbances and for the 52 diabetics without neuropathy are given in table IV. The table clearly shows that with longer duration of diabetes the risk of motor defects is greater for the two younger groups. It also suggests that this risk might be greater when diabetes sets in early.

Mean angiopathy indexes, that is the mean number per patient calculated in accordance with table II, are given in table V for patients with EMG evidence of motor defects and patients without neuropathy. There is evidently a strong correlation between diabetic angiopathy

Table VI reveals that abnormal EMG patterns were recorded from M. extensor digitorum brevis of all patients with motor defects and with diminishing frequency from the M. tibialis anterior and M. interosseus I dorsalis manus Among the 55 patients with EMG changes in M. extensor digitorum brevis 3. showed no activity whatsoever or merely single potentials per needle site. Denervation potentials were recorded from only 7 of the 76 patients with neuropathy extensive paralysis and atrophy being present in all these 7 cases.

The EMG findings agreed well with other clinical evidence of motor defects. Thus the large majority of patients with EMG abnormalities in M. extensor digitorum brevis exhibited signs of atrophy of this muscle, sometimes distinctly visible, sometimes palpable as a flaccid consistency of the contracted muscle. In 10 patients the M. extensor digitorum brevis was completely atrophied.

Conduction velocity

The mean conduction velocity in the ulnar nerve was 58 m/sec. In non-diabetic subjects, 50 m/sec. in diabetics without neuropathy and 49 m/sec. in diabetics with neuropathy If 50 m/sec. is chosen as an arbitrary lower limit of normal ulnar conduction velocity 3 of 28 (11) of th non-diabetics, 8 of 19 (4) of the diabetics without neuropathy and 18 of 27 (67) of the diabet ics with neuropathy would fall below this value. The mean peroneal conduction velocities for non-diabetics and for diabetics without and with neuropathy were 43 38 and 37 m/sec. respectively Supposing the lower limit of normal peroneal conduction velocity were 40 m/sec., then with this arbitrary limit the observed conduction velocities would have been subnormal in 3 of 26 non-diabetics (12 %) 12 of 19 diabetics without neuropathy (63 %) and 18 of 24 diabetics with neuropathy (75 %)

Table III Diabetics with clinical neuropathy classified within age groups by type of nervous involvement

Age group (yrs)	Type of clinical nerv. defect		EMG only	Total
	Sensory + motor	Sensory only		
> 60	28	8	2	38
40–60	16	8	4	28
< 40	3	5	2	10
Total	47	21	8	76

Table IV Mean duration in years within age groups among diabetics with motor defects only and diabetics without neuropathy

Age group (yrs)	Mean duration of diabetes in years	
	Motor defects only	Without neuropathy
> 60	10	11
40–60	13	7
< 40	14	6

Table V Mean index of angiopathic degree within age groups of diabetics with motor defects only and diabetics without neuropathy

Age group (yrs)	Mean index of angiopathic degree	
	Motor defects only	Without neuropathy
> 60	2.4	1.2
40–60	2.4	1.0
< 40	1.6	0.4

Table VI Diabetic patients with neuropathy classified within age groups according to muscle with abnormal responses

Age group (yrs)	No. of patients with abnormal EMG from muscles			Total EMG defects	Total neuropathies
	Ext dig. brevis	Tibialis ant	Int. I dors. manus		
> 60	30	15	5	30	38
40–60	20	7	4	20	28
< 40	5	4	1	5	10
Total	55	26	10	55	76

35 with zero activity or occasional potentials

When the diabetics with clinically manifest neuropathy were divided into a group with and another without EMG evidence of motor defects it appeared that the two groups showed similar mean ulnar peroneal conduction velocities

Discussion

It is interesting to note that positive correlations found in a previous investigation (2) between diabetic neuropathy predominantly without motor defects, and duration of diabetes and also between the former and the index of severity of diabetic angiopathy were verified in the present study on diabetic neuropathy of the motor type.

On the whole we are of the opinion that EMG studies constitute a good complement to ordinary clinical examinations With few exceptions the EMG changes were bilateral Considering that atrophy of M extensor digitorum brevis is the most common motor defect in intervertebral disk herniation involving dorsal root L5 (4) we questioned all our patients about sciatic complaints. Two of them had had severe sciatic pains in one leg and exhibited ipsilateral atrophy of this muscle. They were eliminated from the investigation. Two other patients showed EMG signs of peripheral neurone injury elsewhere, one of them from the eye muscle in oculomotor paresis and the other — who had urinary incontinence — from the external striated sphincter of the urethra.

The mechanism responsible for the high incidence of abnormal EMGs recorded from M extensor digitorum brevis is unclear However a reasonable explanation could be that long nerve fibres are more vulnerable than short ones. This would be in agreement with observations made in experimental ischemia induced in limbs with the aid of a sphygmomanometer cuff (5 7)

In general terms it may be said that the EMG studies in the present investigation disclosed that motor defects are common in diabetics with neuropathy and increase in frequency with the duration of diabetes as well as with the degree of diabetic angiopathy This roughly bears out recent findings re-

orted by Mulder et al. (8) Among the 03 unselected diabetics they studied 33 ad both clinical and EMG changes, 5 nly clinical changes and 5 only EMG abnormalities. Moreover EMG studies can disclose subclinical motor defects and provide objective information on any improvements or exacerbations in the patient neuromotor status. The present investigation reveals that it is most important to examine M. extensor digitorum brevis.

Our measurements of conduction velocity in motor nerves suggest that neuropathy is not a prerequisite for a reduced conduction velocity in diabetics. Similar reductions of conduction velocities have also been observed in other series of diabetics without signs of neuropathy as appears from five investigations published during the past two years (1 3 6 8 9) EMG studies and conduction velocity determinations evidently provide different and complementary data regarding functional changes in the peripheral nerves. Whereas EMG abnormalities may be due to and provide information on inactivation of motor fibres in the peripheral nerve, conduction velocity measurements inform us about the function of the most rapidly conducting intact motor fibres. A reduced conduction velocity may be due either to selective injury to the fastest fibres or to a retarded conduction velocity in all fibres owing to metabolic abnormality However selective inactivation of fast motor fibres appears to be an unlikely though not impossible, cause of the reduced conduction velocity in a group of diabetics without neurological signs of neuropathy The reduced conduction velocity observed in diabetics with neuropathy might be due either to the neuropathy as such or to those rather great metabolic disturbances which could be the ultimate cause of the neuropathy The quickly developing and reversible reduction of the conduction velocity in experimental alloxan diabetes in animals (Eliasson, cited by Mulder et al. (8)) suggests that the slowed conduction velocity in diabetics might reflect general metabolic disturbances.

The clinical diagnosis of diabetic neuropathy often a difficult and subtle matter can be facilitated by the use of EMG and nerve conduction velocity measurements to provide objective data about otherwise insidious or undetectable symptoms. Additional information can be derived from determinations of conduction velocity in sensory nerves.

Summary

EMG examinations carried out on 128 unselected diabetics revealed that motor disturbances are common in diabetic neuropathy — contradicting modern text books — and also that their frequency rises with the duration of diabetes as well as with the degree of diabetic angiopathy The initial signs of motor involvement are exhibited by M. extensor digitorum brevis.

Determinations of motor nerve conduction velocity in a series of 84 subjects, including 30 diabetics with and 20 without clinically manifest neuropathy showed that the mean conduction velocity is subnormal in diabetics whether or not they have clinical neuropathy

Since both the sensory and the motor defects in diabetic neuropathy are often difficult to ppraise objectively the two procedures described here provide the clinician with effective help in the diagnosis of diabetic neuropathy and in following the results of treatment.

Table VI Diabetic patients with neuropathy classified within age groups according to muscle with abnormal responses

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Micro-electrophoresis of Heparin

By

L. B. Jaques, R. E. Ballieux and C. van Arkel

Wilander (17) in 1938 reported the electrophoresis of heparin using the Tiselius apparatus. Recent moving boundary electrophoresis studies (4 15) have shown heparin preparations to be surprisingly homogenous electrophoretically even when differences were observed in biological activity light-scattering and sedimentation properties. There have been relatively few attempts to make use of the electrophoretic properties of heparin in any routine manner. Ruenits (15) described electrophoresis of heparin on filter paper and Bassouni (5) made use of this in his method for estimating heparin in human blood and tissues. Van Arkel et al. 2) have reported the adaptation of the micro-method of Wieme (16) to mucopolysaccharides. With this adaptation heparin can be seen as a distinct metachromatic band and can be distinguished from other mucopolysaccharides by its higher rate of migration and more pronounced metachromatic color 0.01—0.02 µg of heparin are demonstrable. Combined staining for proteins and mucopolysaccharides shows complete separation of heparin and proteins (e. g. human serum proteins) by the microelectrophoresis. The method is therefor ideal for various studies of heparin. The present communication presents the results of an examination of various heparin preparations by this method.

Methods

Electrophoresis was performed on microscope slides coated with gel of agarose (the sulphate-free component of agar) made up in barbiturate buffer pH 8.6 0.001—0.003 ml of the heparin solutions was pplied either to three cuts, 4 mm long, or to two cuts, 5 mm long. Electrophoresis time was 7 min. t a oltage of 20 V/cm, and temperature 11 C. The agar-gel strip was fixed immediately in 0.1 Cetavlon, covered with filter-paper and dried t 37° C, stained for 15 min. in toluidine blue and rinsed in 1 % acetic acid. Some slides were then also stained for protein with Lissamine green as described by van Arkel et al. (2) The density of the red-purple spots in cases of metachromatic staining wer read in a Chromoscan densitometer from Joyce Loebl and Company Ltd., using an Ilford 634 filter. Under these conditions the reference heparin solution (1 mg/ml heparin

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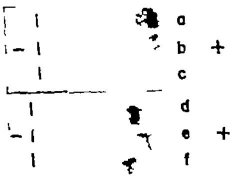


Fig 1 Micro-electrophoresis on agarose gel of commercial heparin a, 10 mg Leo, b, 1 mg Leo, c, 0.1 mg Leo, d, 1 mg Abbott Na heparin, e, 1 mg Leo heparin, f, 1 mg Abbott Ba heparin. Application slots have been underlined. Amounts of heparin pro ml.

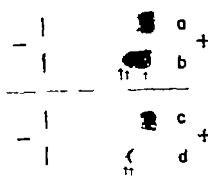


Fig. 2 Micro-electrophoresis on agarose gel of heparin from urinary concentrates b, patient 931 2, a, reference heparin Leo d, normal urine 931-4 c, reference heparin Leo. Lower slide also stained with Lissamine green for protein. Application slots have been underlined.

curred. When different concentrations of heparin were pplied (fig. 1 — a, b, c) the size and more particularly the density of the band corresponded to the concentration of heparin, but when heparin samples from different sources were applied in the same concentration (fig 1 — d, e, f) the optical density was about the same but the position of th band (rate of migration) was different. When urinary concentrates from heparinized patients were ppl ed to the agarose (fig 2b) three bands appeared. One large, prominent band was in the same position as the reference heparin Leo (fig 2a) The other two bands appeared in the urinary concentrat from normal urine (fig 2d) The dark-blue crescent between the two metachromatic bands tended to block out these bands. Urinary concentrates of heparinized patients, which had been precipitated with cetic acid (fig 3c) no longer bowed the middle band The two metachromatic bands thus seen (fig 3c) were also evident in th micro-electrophoresis of heparin treated with heparinase (fig 3a)

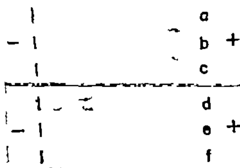


Fig 3. Micro-electrophoresis on agarose gel of heparin incubated with heparinase and urinary concentrate from heparinized patient and d — heparin-heparinase, b and — reference heparin Leo, and f — urinary concentrate 931 2 after precipitation with acetic acid. Lower slide also stained with Lissamine green for protein. Application slots have been underlined.

A solution of 1 mg heparin Leo/ml was pplied to each slide as a reference. Migration and optical density of the spots were measured and are reported in table I as the ratios of the distance and of the area of heparin sample to that of the reference sample. Heparin Leo was

Leo) travelled 2 75 to 3 5 cm and gave a band of 0.14—0.20 cm with slight trailing

Agarose was prepared from Difco Noble agar using the method of Araki (1 6) The agarose was dissolved in barbiturate buffer (10.4 g sodium barbiturate and 1 84 g barbituric acid in 1 000 ml distilled water pH 8 6) Cetavlon was prepared in distilled water 40 mg Toluidine blue was dissolved in 20 ml water and 80 ml of dry acetone. 300 mg Lissamine green was dissolved in 100 ml 1 % acetic acid.

Heparin preparations used

Heparin Leo 5 000 i.u /ml or about 50 mg/ml.

Organon heparin 5 000 i.u./ml or about 50 mg/ml

International standard heparin No. 2 obtained from the Department of National Health and Welfare, Ottawa, Canada, was prepared as a solution of 0 1 mg/ml of saline containing 0.3 % cresol. Defined potency = 130 i.u./mg

Sodium heparinate Abbott and barium heparinate Abbott 92 and 87 i.u /mg respectively (Howell assay) supplied through the courtesy of Dr A. Jones, Abbott Laboratories, Research Division, North Chicago, Ill.

Dog heparin, prepared as the sodium salt We are indebted to Dr S Sasaki for the final stages of this preparation. Potency (in u /mg determined against International Standard powder) — 302 (Howell) 198 (U S P) 138 (Metachromatic)

Beta heparin, supplied by the late Dr O Wintersteın Hoffman-La Roche Ltd Basel

Heparin monosulphuric acid supplied by Professor E. Jorpes, Stockholm.

Urine concentrates prepared by the method of P Astrup (3) A portion of a 24-hour sample of urine was precipitated with benzidine The precipitate in 10 ammonia was extracted with ether and the aqueous phase precipitated with alcohol-sodium chloride. The resulting precipitate was washed with alcohol and ether and dried. Further purification could be achieved by dissolving in a small volume of water and precipitating with nine volumes of acetic acid. Urine was collected from five patients in the University Hospital, Utrecht, a normal subject and two cases of hyperheparinemia. The proved case of hyperheparinemia (H) was reported by Quick (14) in 1957 and has had a continuous history of the condition for many years, including the period following the collection. The other case (U 2) was diagnosed as possible hyperheparinemia in 1959 On returning to the clinic in 1962, there was now no evidence of hyperheparinemia.

Amounts of heparin received by the patients

Urine sample	*Amount of heparin daily administered*
931 1 (patient Va)	2×25 mg+2×50 mg
931 2 (patient de P)	4×50 mg
931 3 (patient Kr)	4×50 mg
931-4 (normal L)	Nil
931 5 (patient Ty)	4×40 mg } mixed 931 5/6
931-6 (patient Ip)	4×50 mg } mixed 931 5/6
931 7 (normal L)	50 mg heparin added to 310 ml urine.

Heparinase was prepared by the original method of Jaques (7) To 18.5 ml of crude enzyme preparation at pH 5 06 was added 8 ml (1 032 i.u.) of heparin in 22 ml of saline tricresol. After being agitated for 24 hours at 34 C, 18 ml of the mixture was lyophilized. The remainder was adjusted to pH 8.8 and assayed on cat blood. It contained 15.6 i u./ml (40 % loss of activity) Micro-electrophoresis was carried out on suspensions of the lyophilized material, of which 100 mg was equivalent to 0 55 mg of heparin added to the original mixture.

Results

Heparin and mucopolysaccharide preparations were applied to the agarose slides at a concentration of about 1 mg/ml for purified mucopolysacchrides, 125 mg/ml for the concentrates from urine, 100 mg/ml for the lyophilized heparinase heparin mixture. Typical agarose slides after micro-electrophoresis, fixing and staining are shown in figs. 1 2 and 3 The dark patches corresponding to the uptake of the dye by the mucopolysaccharide are a bright reddish-purple in the original slide. Rapid migration of heparin has oc

electrophoresis data for urinary concentrates prepared by method of Astrup (3) Leo, 1 mg/ml in water)

Heparin received (mg/24 hrs)	Relative distance traelled	Relative optical density
150	0.77 0.85, 1.01	0.08 0.40, 0.35
200	0.71 0.75, 0.96	1.84 } total O D for all bands
200	0.80, 0.99	2.36 } total O D for all bands
200	0.72, 0.79 0.93	0.59 } total O D for all bands
0	0.72, 0.85	0.63, 0.52 (blue)
0	0.72, 0.82	0.03, 0.07
0	0.75, 0.79	0.04, 0.21
1 mg/6 ml urine	0.99	1.0

...ed patients, re... ...ing the slides of ...ixture for pro... revealed zones ...plication spot. ...seen another ...etachromatic ...ped, stained ...purple, and ...ntrate was ... This band ... other two ... density is ... w for the ...-moving ...n in the ... hyper... ...le from ...a, the ...rom ... of only ... conc... were ... three ban... band ... the reference ... other two b... concentrat... 3d) The dark-... the two metachrom... to block out their ... centrates of heparinuri... had been precipitated (fig. 3c) no longer show... band. The two metachr... seen (fig. 3c) were also ... micro-electrophoresis of h... with heparinase (fig. 3e) ...

the results agree with conclusions regarding heparin reached by other experimental approaches. The fastest migration rate was shown by the two commercial samples of beef heparin (Leo and Organon) Dog heparin has 2 1/2 times the anticoagulant potency of beef heparin. It showed a much slower rate of migration, as did the two samples of Abbott beef heparin. There was no significant difference between the Abbott sodium heparinate and Abbott barium heparinate, their relative position being reversed from one run to another As the dog heparin and Abbott heparin were prepared probably by a similar type of extraction process, and those of Leo and Organon by a different type of process, this suggests that the rate of migration of heparin on electrophoresis depends on the commercial source i e. the method of extraction of heparin from tissue. This has been shown by Jaques and Bell by paper chromatography (8, 9) to be a factor ... the chemical composition of heparin. The indication that the international standard heparin has two

Leo) travelled 2.75 to 3.5 cm and gave a band of 0 14—0.20 cm with slight trailing

Agarose was prepared from Difco Noble agar using the method of Arakı (1 6) The agarose was dissolved in barbiturate buffer (10 4 g sodium barbiturate and 1 84 g bar bituric acid in 1 000 ml distilled water pH 8.6) Cetavlon was prepared in distilled water 40 mg Toluidine blue was dissolved in 20 ml water and 80 ml of dry acetone. 300 mg Lissamine green was dissolved in 100 ml 1 % acetic acid.

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Heparinase was prepared by the original method of Jaques (7) To 18.5 ml of crude enzyme preparation at pH 5.06 was added 8 ml (1 032 i.u.) of heparin in 22 ml of saline tricresol. After being agitated for 24 hours at 34 C, 18 ml of the mixture was lyophilized. The remainder was adjusted to pH 8.8 and assayed on cat blood It contained 15.6 i.u./ml (40 loss of activity) Micro-electrophoresis was carried out on suspensions of the lyophilized material, of which 100 mg was equivalent to 0 55 mg of heparin added to the original mixture.

Results

Heparin and mucopolysaccharide preparations were applied to the agarose slides at a concentration of about 1 mg/ml for purified mucopolysacchrides, 125 mg/ml for the concentrates from urine, 100 mg/ml for the lyophilized heparinase-heparin mixture. Typical agarose slides after micro-electrophoresis, fixing and staining are shown in figs. 1 2 and 3 The dark patches corresponding to the uptake of the dye by the mucopolysaccharide are a bright reddish purple in the original slide. Rapid migration of heparin has oc

Table II Micro-electrophoresis data for urinary concentrates prepared by method of Astrup (3) (Reference heparin Leo, 1 mg/ml in water)

Urine concentrates	Heparin received (mg/24 hrs)	Relative distance travelled	Relative optical density
931—1	150	0.77 0.85, 1.01	0.06 0.40, 0.35
931—2	200	0.71 0.75, 0.96	1.84 } total O. D for all bands
931—3	200	0.80, 0.99	2.56
931—5/6	200	0.72, 0.79, 0.93	0.59
Hyperheparinemia			
H	0	0.72, 0.85	0.63, 0.[illegible] (blue)
U-2 (in remission)	0	0.72, 0.82	0.05, 0.07
Normal controls			
931—4	0	0.75, 0.79	0.04 0.21
931—7 + added heparin	1 mg/8 ml urine	0.99	1.0

centrates from heparinized patients, reported in table II Staining the slides of the heparin-heparinase mixture for proteins with Lissamine green revealed zones of proteins closer to the application spot. In th urine concentrates is seen another band between the two metachromatic bands. This was crescent-shaped, stained blue-purple rather than red purple, nd disappeared when the concentrate was precipitated w th ceti acid. This band in many samples overlaps the other two and therefore the total optical density is reported with no attempt to allow for the overlap. This band and the slow-moving metachromatic band alone are seen in the urine samples from the normal and hyper heparinemia patients In the sampl from the case of active hyperheparinemia, the band of slowest mobility is more prominent than in other cases

Discussion

The different electrophoretic mobilities of heparin observed by this technique certainly require explanation. Fortunately the results agree with conclusions regard ing heparin reached by other experimental approaches. The fastest migration rate was shown by the two commercial samples of beef heparin (Leo and Orga non) Dog heparin has 2 1/2 times the anticoagulant potency of beef heparin. It showed a much slower rate of migra tion, as did the two samples of Abbott beef heparin. There was no significant difference between the Abbott sodium heparinate and Abbott barium heparinate, their relative position being reversed from one run to another As the dog heparin and Abbott heparin were prepared probably by a similar type of extraction process, and those of Leo and Organon by a different type of process, this suggests that the rat of migration of heparin on electrophoresis depends on the commercial source, i. e. the method of extraction of heparin from tissue. This has been shown by Jaques and Bell by paper chromatography (8, 9) to be a factor in the chemical composition of heparin. The indication that the international standard heparin has two

Table I Micro-electrophoresis data for heparin and related compounds (Reference heparin Leo, 1 mg/ml in water)

Sample	Concentration tested (mg/ml)	Relative distance travelled	Relative optical density
Heparin preparations			
Reference heparin Leo	1.0	1.00	1.00
Heparin Leo	10	1 00	1.92
Heparin Leo	0.5	1.00	0.25
Heparin Leo	0.1	0.995	0.01
International standard	0.1	0.88, 0.97	0.16
Heparin organon	1 00	1.00	0.44
Abbott sodium heparin	1 00	0.864	1.20
Abbott barium heparin	1 00	0.884	1 10
Dog heparin	1.00	0.902	0.645
Mucopolysaccharides			
Chondroitin sulphuric acid C (β-heparin)	1.00	0.867	0.404
Chondroitin sulphuric acid A	1 00	0.705	(blue-purple)
Heparin monosulphuric acid	1 00	?	(blue-purple)
Hyaluronic acid	1 00	0.500	0.00
Heparinase			
+ Heparin added	0.55	0.82, 0.94	0.27 0.19

applied in different concentrations. For the 1 mg/ml concentration the migration and optical density were measured for the reference solution on five slides and the ratio of the mean values to that of the reference used for Lo and 0 1 mg/ml determined. The ratio with standard deviation was 1 11 ± 0 169 for the migration distance and 1 11 ± 0 318 for the optical density Evidently reproducibility of values for migration and optical density is satisfactory Optical density as measured by the chromoscan appears to change as the logarithm of the concentration of the heparin-solution Heparin Organon showed the same rate of migration as heparin Leo used as reference However the Abbott heparins and dog heparin showed a slower migration than the heparin Leo reference. International standard heparin showed two faint bands with these migration values. Still slower migrations were shown not only by beta heparin and chondroitin sulphuric acid, but also by some of the samples from urine Heparin monosulphuric acid did not give a band There was irregular blue purple staining at a relative distance of 0 73 to 0 94 from the point of application Chondroitin sulphuric acid gave a blue-purple crescent-shaped band The staining with this and with heparin monosulphuric acid was distinctly different from that of heparin.

While single metachromatic bands are shown for the heparin samples, after treatment with heparinase two bands are seen with mobilities, 0 81 and 0.91 of that of the reference heparin Leo These bands can also be seen in the urine con

and as these samples contained material which could be used as internal references (heparin Leo, chondroitin sulphuric acid) electrophoretic mobility could be used to distinguish the components.

For the individual urine samples, sample 931 7 the normal urine with heparin added shows a strong band with fast migration at the same speed as the reference heparin. This is evidently the heparin Leo added to the urine. Sample 931-4 the sample from normal urine, and sample U_w from essentially normal urine, shows two bands, both of which migrate more slowly than the reference heparin. The more prominent is chondroitin sulphuric acid. The weaker slower band is that of uroheparin. Three bands are seen with the concentrates from urine of heparinized patients. The fastest component corresponds to the heparin Leo injected, the slowest to uroheparin and the intermediate spot, chondroitin sulphate. Patient U shows the same excretion as the normal individual. However the case of hyperheparinemia (sample H) ppears to show a greater excretion of the two slow-moving components. The band for uroheparin showed a much greater optical density than in normal urine but was more blue than that observed with the other samples. This is at present under further investigation.

Summary

Heparin from various sources was subjected to micro-electrophoresis on garose gel and identified by metachromatic staining with toluidine blue. Commercial heparin showed only a single component but the rate of migration depended on the commercial source (method of extraction used) Dog heparin showed the same electrophoretic mobility as beef heparin. After treatment with heparinase, a second more slowly moving component was observed (uroheparin) In urine of heparinized patients, both these components could be identified, as well as a third material, less metachromatic (chondroitin sulphuric acid) Urine from normal individuals and from a case of hyperheparinemia showed two mucopolysaccharide components, corresponding to uroheparin and chondroitin sulphuric acid.

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electrophoretic components is in line with this, since the standard was prepared by mixing heparin prepared by different manufacturers Paper chromatography shows that other mucosaccharides and polysaccharides are present in heparin preparations in close association (by hydrogen bonding?) The net charge of the total complex ion and hence its speed of migration electrophoretically will probably depend on the other molecules included in its structure. The marked homogeneity of electrophoretic behaviour of heparin by the Tiselius procedure reported by Barlow et al. (4) and Laurent (13) is probably due to the heparin being from the same source. Laurent used a single original preparation Barlow et al. did compare beef and pork heparin as the streptomycin complex, and found a difference in electrophoretic pattern.

Definitely slower migration is shown by chondroitin sulphuric acid C (beta heparin) chondroitin sulphuric acid heparitin mono-sulphuric acid, and hyaluronic acid The last shows very little staining with the procedure used

In the case of the heparinase heparin mixture, there are two metachromatic zones and also as there is considerable protein in the tissue extract zones for proteins with the Lissamine green stain The latter were much closer to the zone of application, and there was no overlap indicating complete separation of the protein and mucopolysaccharide at the pH used for electrophoresis. For the two mucopolysaccharide spots, one corresponds to that for Abbott heparin and is evidently the original heparin added to the tissue extract. There is also present a much more slowly moving component, which corresponds to the component in the urine concentrates described below

As Jaques and Jaques (10) have recently also demonstrated by assay procedures that uroheparin is the product of the action of heparinase, this is evidently the spot for uroheparin (11)

In the urine concentrates from benzidine precipitation, three components were observed by micro-electrophoresis. These showed average relative mobilities, compared to the reference heparin, of 0 735 0 817 0.978 Both in the patterns observed with the mucopolysaccharides already described and in those observed with the urine concentrates three components can be clearly identified. The fast component from position and staining properties is evidently unchanged heparin. The weaker slow band also shows red color Its position corresponds to that seen for uroheparin with heparinase, so it is evidently uroheparin The middle component can be distinguished by the nature of its staining with toluidine blue, since it gave a blue purple crescent in the urine concentrates, as with authentic chondroitin sulphuric acid This could be clearly distinguished from the reddish-purple bands observed for the slow and fast components of the urine concentrates and heparinase-treated heparin and for the single component of authentic heparin. It could also be distinguished by the fact it could be removed from the material with acetic acid (cf fig 2c and fig 3c) The middle component is evidently a chondroitin sulphuric acid. As quite different preparative methods and means of identification were used it is not possible to correlate further these observations with those of King et al (12) The results with the heparin samples (table I) indicate that the electrophoretic migration of heparin depends on the extraction procedure but the same extraction procedure was used for all the urine samples,

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Aarhus County Hospital, Aarhus, Denmark

Creatine Phosphokinase in the Diagnosis of Myocardial Infarction

By

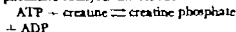
Niels Schwartz Sorensen

The enzyme creatine phosphokinase was described for the first time in 1934 by Lohman (12). The function of the enzyme is closely related to the energy-generating processes on which muscle contraction is dependent. Creatine phosphokinase catalyzes the reaction

$$ATP + creatine \rightleftharpoons creatine\ phosphate + ADP$$

The chemical and kinetic properties of the enzyme have been studied in detail by several investigators (5, 9, 11, 14).

Creatine phosphokinase is found in a high concentration in striated muscular tissue and in a somewhat lower concentration in cardiac muscular tissue and in brain tissue. The concentration in the tissues of the lung, liver, kidney and pancreas is almost zero (4, 13, 16, 17, 18).



As creatine phosphokinase is almost exclusively a muscle enzyme, the determination of the activity of this enzyme in serum (S-CPK) might well be of interest in certain diseases involving muscular tissue. This assumption has been confirmed during recent years, as it has been possible to show elevated activities in the serum from patients suffering from various muscle diseases and myocardial infarction. The first report on the use of determinations of S-CPK as a diagnostic aid was published by Ebashi et al. (8). These investigators demonstrated increased values of S-CPK in patients with progressive muscular dystrophy. This finding has been confirmed by others (4, 6, 15). Increased activity of S-CPK has also been demonstrated in other primary muscle diseases, but not in secondary neurogenic muscle diseases. Some of the highest activities have been found in patients with dermatomyositis (4, 15). Increased values of S-CPK in patients with myocardial infarction were first demonstrated by Dreyfus et al. (7). This has also been confirmed by other investigators (4, 10, 18).

The determination of the activity of the enzyme can be made according to various principles, as it is possible to

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The specific molar extinction coefficient of DPNH at 340 mμ is 6.22 × 10[6] On the basis of this value it is possible to calculate the absolute changes in the concentration of DPNH.

Reliability of the method

The reliability of the method was estimated on the basis of 41 duplicate determinations (82 single determinations). Sixteen duplicate determinations showed values below 2 μ Ez/l In this area, the standard deviation of a single analysis (S_a) was found to be 0.1 μ Ez/l ($S_a = \frac{d^2}{2n}$ n = number of duplicate determinations, d = difference between corresponding determinations). In the area between 2 and 20 μ Ez/l S_a was found to be 0.6 μ Ez/l. This estimate was based on the results of 25 duplicate determinations.

Normal values

The range of normal values was established on the basis of determinations of S-CPK in 34 adults. These determinations were made in patients with diseases which are unlikely to exert any influence on S-CPK (depression, degenerative joint disease and patients admitted for observation without any demonstrable disease). Six subjects were under 30 years, one was 84 and the rest between 30 and 70 years.

The results of the determinations of S-CPK in the individuals examined are illustrated in fig. 2. The mean value was found to be 0.07 μ Ez/l. Standard deviation of the mean, S, was calculated as 0.12 μ Ez/l. On the basis of these figures, the normal range of S-CPK was established to be less than 0.3 μ Ez/l (= mean + 2 S).

Material

The series consisted of patients who were admitted with suspected coronary occlusion. The determinations of S-CPK were usually carried out daily during the first three days after admission. Some of the patients were followed for longer periods. In parallel with S-CPK, determinations of serum glutamic oxaloacetic transaminase (S-GOT) and serum glutamic-pyruvic transaminase (S-GPT) by the method of Karmen et al. were also performed. In addition, electrocardiograms (leads I, II, III, V_1, V, V) were taken, and the sedimentation rate, leukocyte count and blood pressure were determined.

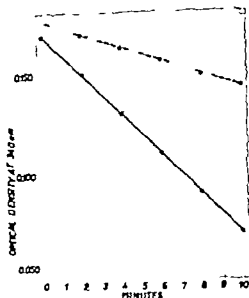


Fig. 1. The fall in optical density at 340 mμ in reaction mixtures without (top) and with creatine (bottom).

The series consisted of 120 patients, who on the basis of the final diagnosis were divided into the following groups

		No. of cases
I	Myocardial infarction	64
II	Doubtful cases	8
III	Arteriosclerotic or hypertensive heart disease	27
IV	Tachycardia	7
V	Pulmonary disease	5
VI	Abdominal disease	4
VII	Miscellaneous diseases	5
	Total	120

I. Myocardial infarction

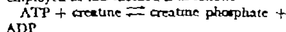
Myocardial infarction was present in 64 patients. Of these, 52 revealed increased values of S-CPK in one or more determinations. The remaining 12 patients all had normal levels of S-CPK. In 11 of these cases, the first determination was performed more than 72 hours

follow variations in the concentrations of one or more of the reagents participating in the reaction (3, 6 8 9 11 13 14 16). Most methods are based on a chemical determination of creatine or creatine phosphate. The method which is now most commonly used was elaborated by Tanzer and Gilvarg (18). The principle applied here is the coupling of two auxiliary reactions to the reaction catalyzed by creatine phosphokinase. The last link in the series of reactions is an indicator reaction which can be followed directly in a spectrophotometer. A more detailed description is given below.

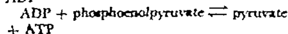
The study reported here was performed in an attempt to evaluate the usefulness of determinations of S-CPK in the diagnosis of myocardial infarction.

Methods

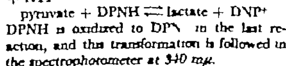
S-CPK was determined by the method of *Tanzer and Gilvarg (18) with slight modifications.* The complete system of reactions employed in this method is as follows



ATP + creatine ⇌ creatine phosphate + ADP



ADP + phosphoenolpyruvate ⇌ pyruvate + ATP



pyruvate + DPNH ⇌ lactate + DNP+

DPNH is oxidized to DPN in the last reaction, and this transformation is followed in the spectrophotometer at 340 mμ.

Pyruvate present in the serum will participate in the reactions. Furthermore the splitting of phosphoenolpyruvate to pyruvate induced by serum alkaline phosphatase must be taken into account. This pyruvate obviously also participates in the reaction. Finally the presence of ATP ase in the serum cannot always be excluded. This enzyme will cause a splitting of ATP to ADP which will then be transformed in the system of reactions. On account of these circumstances a change in the concentration of DPNH will always take place when serum is added to the system, and it is therefore necessary to carry out a determination with a *blank* in which creatine is excluded from the reaction mixture. The difference between these two determinations allows of a calculation of the activity of creatine phosphokinase.

Reagents

I ATP and DPNH in 2 M glycine buffer pH 9. Concentration of DPNH 10^{-3} M. *Concentration of ATP* 9×10^{-3} *M*

II Phosphoenolpyruvate and $MgSO_4$ in distilled water. Concentration of phosphoenolpyruvate 10^{-2} M. Concentration of $MgSO_4$ 1.1×10^{-1} M

III Pyruvate kinase and lactic dehydrogenase in distilled water. 2 mg enzyme protein of each enzyme is dissolved per ml water

IV Creatine in 0.1 M glycine buffer pH 9. Concentration of creatine 8.8×10^{-2} M

V *0.1 M glycine buffer pH 9*

ATP DPNH phosphoenolpyruvate pyruvate kinase and lactic dehydrogenase were supplied by "Boehringer

Procedure

Into each of two test tubes are pipetted 1 ml serum, 0.5 ml reagent I 0.15 ml reagent II and 0.05 ml reagent III. The reagents are mixed thoroughly and the two test tubes are placed in a water bath at 25 C for 15 min. Within this time pyruvate present in serum will be consumed. The final step in the determination is then started by adding 1.3 ml of reagent IV to one of the test tubes and 1.3 ml reagent V *to the other test tube. After* thorough mixing of the reagents, the reactions in the two mixtures are followed in a spectrophotometer at 340 mμ. The readings are made against a reference which contains a *convenient amount of* DPNH. The changes in optical density are measured every minute for 10—12 minutes. Fig. 1 illustrates the results of a determination as described.

Enzyme unit

One unit of the enzyme creatine phosphokinase $Ex\frac{\text{mol/min.}}{\text{own method}}$ is defined as the amount of enzyme, which under the conditions employed induces a phosphorylation of 1 mol substrate (creatine) or in the test system, induces a conversion of 1 mol DPNH to DPN.

The concentration of the enzyme in serum is given as μ Ex/1 serum.

Table I. S-CPK, S-GOT and S-GPT in 26 patients with myocardial infarction. Normal values: S-CPK < 0.3 µE/ml, S-GOT < 25 Karmen units/ml, S-GPT < 20 Karmen units/ml. The patients were all followed from the first day of the disease until the value of S-CPK had again returned to normal or until the death of the patient

Case no.	1st day of disease			2nd day of disease			3rd day of disease			4th day of disease			Death
	S-CPK	S-GOT	S-GPT	S-CPK	S-GOT	S-GPT	S-CPK	S-GOT	S-GPT	S-CPK	S-GOT	S-GPT	
1	1.1	116	44	0.6	55	29	0	37	32	—	—	—	—
2	0.6	39	16	0.2	34	16	—	—	—	—	—	—	—
3	0.6	35	14	0	26	13	0	20	13	—	—	—	—
4	0.7	23	29	0.3	24	27	0	15	24	—	—	—	—
5	0.7	53	12	0.2	44	11	0	32	11	—	—	—	—
6	1.0–4.2	31	14	2.1	33	46	1.1	250	43	0.7	140	42	—
7	1.6	145	63	0.4	73	46	0	42	38	—	—	—	—
8	1.7	155	21	0.8	150	24	0.1	87	17	—	—	—	—
9	0.5	28	12	0.6	33	13	0.7	43	16	0	40	15	—
10	0.6	43	17	0.7	88	20	0	45	21	—	—	—	—
11	1.7	137	20	1.0	214	34	0.2	88	29	—	—	—	+
12	4.3	209	31	1.6	400	54	0.5	161	31	0.2	53	48	+
13	2.0	40	10	4.5	124	19	1.6	129	29	1.0	84	24	
14	0.8–2.1	29	5	2.2	54	27	2.0	206	33	0.4	86	24	
15	0.8	125	55	1.9	169	57	1.0	—	—	—	—	—	+
16	0.5	—	—	0.5	50	16	1.4	227	47	—		—	+
17	0.4	—	—	0.5	24	10	0.5	103	26	—		—	+
18	2.3	101	13	1.7	43	11	0	31	9	—			—
19	0	10	6	1.6	40	9	0.4	40	10	0.5	9	8	—
20	0	63	10	0.5	148	24	0.4	127	36	0	73	38	+
21	0.2	33	5	1.5	40	5	0.2	134	31	—		—	—
22	0.8	19	14	0.1	22	9	0	14	10	—			+
23	0	28	9	0.7	38	10	0	—	—	—	—		—
24	0.7	23	38	0.3	47	37	—	—	—	—	—	—	
25	4.4	180	29	2.0	149	32	0	58	18	—			—
26	1.4	18	10	0	77	19	0.2	56	19	—	—		

showed elevated values of S-CPK on the third day of the disease 40 died. The pertinent data are analysed in table III.

II *Doubtful cases*

This group consists of eight cases in which it was not possible to make an unquestionable diagnosis. The results of the determinations of S-CPK, S-GOT and S-GPT are listed in table IV. The times given in this table are reckoned from the admission to hospital and do not refer to the duration of the disease, since in most cases it was impossible to obtain accurate information as to the onset of the disease. Cases 53, 54 and 55 had normal values for S-CPK, but elevated values of S-GOT and S-GPT. Case 53 had ventricular tachycardia and died. Autopsy was performed, but no signs of myocardial infarction were revealed. The coronary arteries were arteriosclerotic but patent and without thrombosis. The liver was congested. Case 54 did not show any sign of coronary occlusion, apart from increased values of S-GOT and especially S-GPT. Unfortunately the patient was not subjected to further investigations as to the possibility of liver disease. Repeated electrocardiography showed normal conditions. Case 55 complained of precordial pain radiating to the back. This patient had undergone chole-

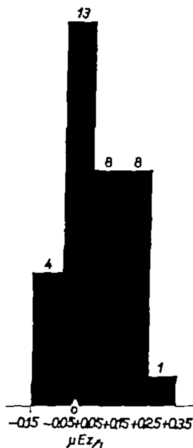


Fig 2 The distribution of the S-CPK values in 34 normal adults.

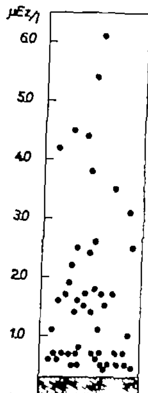


Fig 3 The highest values of S-CPK measured in 52 patients with myocardial infarction. The hatched area indicates the normal range.

after the onset of the disease, while the last patient died suddenly from myocardial infarction 12 hours after the first determination. The S-GOT level was increased in four of the 12 patients and normal in eight.

Figs 1 and 2 show the results of the determinations of S-CPK, S-GOT and S-GPT in the 52 patients with increased values of S-CPK. The highest values of S-CPK measured in the 52 patients are shown in fig 3 However it must be emphasized that the values in fig 3 do not necessarily in all instances represent the actual maxima of S-CPK during the disease as some of the patients were not followed from the onset of the disease. In most cases, the elevation of S-CPK was moderate 38 patients had maximum values below 2 μ Ez/L. The highest value was 6μ Ez/L.

Fig 1 shows the time relation between the onset of infarction and the values of S-CPK. The 26 patients entered here were all followed from the first day of the disease until the value of S-CPK had again returned to normal or until the death of the patient. Increased values of S-CPK appeared early in 21 patients within the first 24 hours. In cases 3 and 14 increases were observed as early as 3 and 6 hours after the onset of the infarction.

The increased levels of S-CPK returned to normal fairly rapidly Thus, seven patients showed normal values on th second day and normal levels were recorded on the third day in 15 out of 24 patients studied In the entire group (tables I and II) 43 patients were studied on the third day of the disease Of these, 18 revealed normal values of S-CPK.

The S-GOT was not invariably followed until a normal value appeared. However it is beyond doubt that the S-GOT in most cases remains elevated for a longer period than the S-CPK.

Finally it should be mentioned that estimation of S-CPK may be of prognostic value. It seems as if the persistence of increased S-CPK for more than 48 hours indicates a relatively bad prognosis. Of the patients who

Table I. S-CPK, S-GOT and S-GPT in 26 patients with myocardial infarction. Normal values: S-CPK < 0.3 μE/l, S-GOT < 25 Karmen units/ml, S-GPT < 20 Karmen units/ml. The patients were all followed from the first day of the disease until the value of S-CPK had again returned to normal or until the death of the patient

Case no.	1st day of disease			2nd day of disease			3rd day of disease			4th day of disease			Death
	S-CPK	S-GOT	S-GPT	S-CPK	S-GOT	S-GPT	S-CPK	S-GOT	S-GPT	S-CPK	S-GOT	S-GPT	
1	1.1	116	44	0.6	55	29	0	37	32	—	—	—	—
2	0.6	39	16	0.2	34	16	—	—	—	—	—	—	—
3	0.6	33	14	0	26	15	0	20	13	—	—	—	—
4	0.7	23	29	0.3	24	27	0	15	24	—	—	—	—
5	0.7	53	12	0.2	44	11	0	32	11	—	—	—	—
6	1.0–4.2	31	14	2.1	33	46	1.1	230	43	0.7	140	42	—
7	1.6	143	65	0.4	73	46	0	42	38	—	—	—	—
8	1.7	155	21	0.8	150	24	0.1	87	17	—	—	—	—
9	0.5	28	12	0.6	53	13	0.7	43	16	0	40	13	—
10	0.6	43	17	0.7	88	20	0	45	21	—	—	—	—
11	1.7	137	20	1.8	214	34	0.2	68	29	—			+
12	6.3	209	31	1.6	400	34	0.3	161	31	0.2	55	48	
13	2.8	40	10	4.5	124	19	1.6	129	29	1.0	84	24	—
14	0.8–2.1	29	5	2.2	34	27	2.8	206	33	0.4	86	24	
15	0.8	125	55	1.9	169	57	1.0	—		—	—	—	
16	0.3	—	—	0.5	50	16	1.4	227	47	—			+
17	0.4	—	—	0.5	24	10	0.5	105	26	—		—	+
18	2.5	101	13	1.7	43	11	0	31	9	—	—		—
19	0	10	6	1.6	40	9	0.4	40	10	0.5	9	8	—
20	0	63	10	0.5	148	24	0.4	127	36	0	73	38	
21	0.2	33	3	1.5	40	3	0.2	134	31		—		
22	0.8	19	14	0.1	22	9	0	14	10				+
23	0	26	9	0.7	38	10	0						—
24	0.7	23	38	6.3	47	37	—	—		—			
25	4.4	180	39	2.0	149	32	0	58	18	—			
26	1.4	18	16	0	77	19	0.2	56	19				

showed elevated values of S-CPK on the third day of the disease. 40 ... died. The pertinent data are analyzed in table III.

II. *Doubtful cases*

This group consists of eight cases in which it was not possible to make an unquestionable diagnosis. The results of the determinations of S-CPK, S-GOT and S-GPT are listed in table IV. The times given in this table are reckoned from the admission to hospital and do not refer to the duration of the disease, since in most cases it was impossible to obtain accurate information as to the onset of the disease. Cases 53, 54 and 55 had normal values for S-CPK, but elevated values of S-GOT and S-GPT. Case 53 had ventricular tachycardia and died. Autopsy was performed, but no signs of myocardial infarction were revealed. The coronary arteries were arteriosclerotic but patent and without thrombosis. The liver was congested. Case 54 did not show any signs of coronary occlusion, apart from increased values of S-GOT and especially S-GPT. Unfortunately the patient was not subjected to further investigations as to the possibility of liver disease. Repeated electrocardiography showed normal conditions. Case 55 complained of precordial pain radiating to the

Table II S-CPK S-GOT and S-GPT in 26 patients with myocardial infarction Normal values S-CPK < 0.3 μEz/l, S-GOT < 25 Karmen units/ml, S-GPT < 20 Karmen units/ml

Case no.	1st day of disease			2nd day of disease			3rd day of disease			4th day of disease			Death
	S-CPK	S-GOT	S-GPT	S-CPK	S-GOT	S-GPT	S-CPK	S-GOT	S-GPT	S-CPK	S-GOT	S-GPT	
27	—	79	11	2.2	60	13	0.5	18	7	—	—	—	—
28	0.4	14	5	1.7	84	17	2.4	89	22	—	—	—	—
29	3.8	84	15	1.8	237	44	1.9	143	26	—	—	—	—
30	0.2	11	6	0.5	50	7	5.4	29	31	—	—	—	—
31	1.8	176	24	1.2	195	30	—	118	23	—	—	—	—
32	0.3	17	5	2.6	58	14	1.6	76	20	—	—	—	—
33	0.6	34	13	—	30	15	—	24	13	—	—	—	—
34	—	—	—	—	—	—	1.1	44	29	0	29	25	—
35	—	—	—	—	183	29	0.7	164	41	—	103	52	—
36	—	—	—	—	—	—	0.5	64	16	0.3	45	11	—
37	—	—	—	—	—	—	0.5	43	18	0.4	40	18	—
38	—	95	17	1.7	105	24	1.6	68	21	—	—	—	—
39	—	—	—	6.1	44	11	—	69	18	—	65	18	—
40	—	21	6	1.5	121	16	0	73	14	—	—	—	—
41	0.4	30	7	—	123	16	—	246	23	—	—	—	—
42	—	38	9	1.7	100	9	0.1	42	18	—	—	—	—
43	—	49	21	—	113	23	0.5	65	29	—	—	—	—
44	0.7	26	18	—	—	—	—	—	—	—	—	—	—
45	0	17	8	0.9	101	37	—	88	30	—	—	—	—
46	—	—	—	3.5	172	24	0.6	104	29	0	33	18	—
47	—	—	—	0.5	59	14	0.3	47	14	0	43	13	—
48	—	—	—	—	—	—	0.7	510	158	0	250	912	+
49	—	—	—	—	60	12	1.0	324	31	0	390	128	+
50	0.1	10	6	0.4	8	5	0.5	7	4	—	—	—	+
51	—	150	22	3.1	220	33	1.1	178	27	—	—	—	+
52	—	—	—	2.5	151	34	—	—	—	—	—	—	+

cystectomy in 1955 and had pancreatitis in 1957 During his present stay in hospital duodenal ulcer was demonstrated Intravenous cholangrography did not reveal any abnormalities.

It seems reasonable to assume that the increase in the activity of serum transaminases in these three cases was due to damage to the liver parenchyma, and that none of the patients had myocardial infarction. This might offer an explanation of the normal values of S-CPK and the elevated values of S-GOT and S-GPT However it must be admitted that the determinations of S-CPK may have been performed too late in the disease.

The last five patients all showed elevated values of S-CPK and all but one (case 56) had normal activities of serum transaminases. By a critical evaluation of these cases it was found that all these patients may have suffered from coronary occlusion. Some years previously case 56 had been admitted with coronary occlusion. Death ensued suddenly at home one month after discharge from hospital. Case 57 complained of severe precordial pain. He had an attack 12 hours before the elevated value of S-CPK was revealed. Five electrocardiograms were normal. There was no sign of liver disease. Intravenous pyelography and cholangiography showed normal conditions. Case 58 had an attack of severe precordial pain 3 hours before admission. Blood pressure fell from 140/90 to 105/65 during the hospital stay Electrocardiography was normal, and

no other signs of coronary occlusion were observed. Case 59 had previously suffered from myocardial infarction twice. During the two last days before admission, he had experienced several attacks of precordial pain and had taken 15—25 nitroglycerin tablets daily. The electrocardiogram did not allow of any definite conclusion as to a new episode of infarction. Case 60 suffered from polycythaemia. On the day of admission he had intense precordial pain with radiation to both arms. The electrocardiogram was normal.

It is impossible conclusively to prove that these last five patients suffered from coronary occlusion, but it seems likely that this may be the correct diagnosis. In this connection it is of interest that all patients had elevated S-CPK, but only one (case 56) showed slight elevation of serum transaminases.

III. *Arteriosclerotic or hypertensive heart disease*

Twenty-seven patients observed for coronary occlusion appeared to suffer from arteriosclerotic or hypertensive heart disease without sign of myocardial infarction. All patients had normal values of S-CPK and serum transaminases. Of these patients, 16 were examined within 48 hours after the onset of the symptoms suggesting coronary occlusion.

IV. *Tachycardia*

Seven patients exhibited various forms of tachycardia, but otherwise no signs of coronary occlusion. Four of these showed atrial fibrillation with heart rates between 100 and 150. The activities of all three enzymes were

Table III. The S-CPK level on the third day of myocardial infarction related to mortality

S-CPK level on 3rd day of disease	No. of cases	No. of deaths
Increased	23	10
Normal	18	3
Total	43	13

Total no. of patients with myocardial infarction 64

Total no. of deaths among these patients 16

normal in all patients. One patient had ventricular tachycardia with a rate of 270. This patient showed normal S-CPK, while the S-GOT and S-GPT were slightly elevated. After the attack, a normal electrocardiogram was recorded. Two patients suffered from supraventricular tachycardia. Both had normal values of S-CPK, S-GOT and S-GPT.

V. *Pulmonary disease*

This group consists of five patients. Two patients had chronic cor pulmonale, two infarction of the lung and one acute bronchitis. The activities of the three enzymes were all within normal limits.

VI. *Abdominal disease*

Four patients admitted for coronary occlusion actually suffered from abdominal disorders. Two patients suffered from chole-

Table IV. S-CPK, S-GOT and S-GPT in seven patients with uncertain diagnosis (see text). Normal values: S-CPK 0.3 μE/l, S-GOT < 25 Karmen units/ml, S-GPT 20 Karmen units/ml

Case no.	1st day in hospital			2nd day in hospital			3rd day in hospital		
	S-CPK	S-GOT	S-GPT	S-CPK	S-GOT	S-GPT	S-CPK	S-GOT	S-GPT
53	0.3	172	103	0	68	108	0	74	114
54	0.3	18	15	0.1	27	23	0	39	45
55	0	46	17	0	81	58	0	22	38
56	0.6	23	23	0	33	16	0	28	18
57	0	12	9	0	10	7	0.5	10	7
58	0.7	20	11	0.2	13	7	0	12	9
59	0.7	11	11	0	12	4	0	12	10
60	0	16	7	0.4	13	[illegible]	[illegible]	[illegible]	[illegible]

thiasis both showed increased S-GOT and S-GPT but normal values of S-CPK. One of the patients was jaundiced. Both patients were subjected to operation. Of the remaining two patients, one had ileus and the other hiatus hernia. No abnormality in the enzyme pattern was noticed

VII. *Miscellaneous diseases*

The last five patients in the series suffered from disorders other than those already mentioned. Two patients had cerebral haemorrhage and three had disorders of the thoracic skeleton. No abnormal values of the three enzymes were recorded in these patients.

Discussion

The technical determination of S-CPK is rather simple although not quite so simple to perform as the determination of the serum transaminases. The reason for this is the necessity of carrying out two parallel determinations in the case of S-CPK. However the method of Tanzer and Gilvarg (18) is much easier to perform than most other methods The author has some experience with the method of Ennor and Rosenberg (9) based on the determination of creatine and another method based on the determination of creatine phosphate Both methods are rather troublesome

The results of the determinations of S-CPK in the patients with myocardial infarction seems to indicate that this determination is of definite help in the diagnosis. All patients with coronary occlusion will probably show elevated values if they are examined early enough after the onset of the disease. This appears from the fact that 52 patients with myocardial infarction examined within the first 72 hours after the onset of the disease all exhibited elevated values of S-CPK in one or more determinations. However it must be emphasized that the possibility of demonstrating an increase in S-CPK is much less when more than 48 hours have elapsed since the infarction. This is evident from the results of the examinations of the patients on the third day of the disease, at which time it was found that 18 (40 %) of 43 patients revealed S-CPK values within the normal range.

The question whether increased values of S-CPK can be demonstrated in the absence of myocardial infarction is rather difficult to answer Five patients without a definitely established diagnosis of coronary occlusion showed increased values of S-CPK. In all five patients, the case histories were suggestive of coronary occlusion On account of this, it seems likely that the increased values of S-CPK in these cases were referable to undiagnosed infarction of the myocardium.

In the opinion of the author an increased S-CPK level almost invariably indicates that the patient suffers from myocardial infarction, provided that other types of myopathy especially progressive muscular dystrophy and myositis, can be excluded. It is uncertain whether muscular contusions may provoke an increase in S-CPK Investigations into this problem are in progress.

The most common cause of a false interpretation of the results of the determinations of serum transaminases in cases suspected of coronary occlusion is the presence of disorders of the liver parenchyma. Such disorders may appear coincident with and independent of coronary occlusion (1 2 19) They may also be demonstrated in heart diseases which are not accompanied by myocardial infarction. As there is practically no creatine phosphokinase activity in the

liver hepatic disease is, as might be expected, never accompanied by an increase in S-CPK. This is an unquestionable advantage. The author investigated this phenomenon further by examining seven patients with jaundice. Four of these suffered from cirrhosis of the liver two had epidemic hepatitis and one had cholelithiasis. In all cases, S-CPK was normal, while S-GOT and S-GPT were markedly increased.

In the cases with coronary occlusion, it was seen that S-CPK was increased for a shorter period than S-GOT This relationship is similar to that between S-GOT and serum lactic dehydrogenase. It is well known that the activity of the latter enzyme is increased in the serum for a longer period than that of S-GOT after an episode of myocardial infarction At the present time, it is impossible to offer any explanation of these differences. In practice, however it seems justifiable to assume that simultaneous determinations of S-CPK and lactic dehydrogenase in the serum might provide a valuable additional aid in the diagnosis of coronary occlusion

In order to assess the benefit that might be derived from the determination of S-CPK in patients suspected of coronary occlusion, the author critically analysed the usefulness of the method in the individual cases. A diagnostic aid in cases suspected of coronary occlusion was defined as follow The determination (1) finally established the diagnosis of coronary occlusion at an early stage of the disease, (2) secured this diagnosis in otherwise doubtful cases, or (3) excluded the possibility of coronary occlusion. On the basis of these criteria, it was found that the determination of S-CPK had been helpful in 30 of the 120 cases investigated, i. e. 25 %.

Summary

The enzyme creatine phosphokinase is described and previous papers concerning this enzyme are considered. The use of the determination of S-CPK as an aid in the diagnosis of disorders of muscles and myocardium is mentioned. A description of the technique of the determination of S-CPK used by the author is then given This is a modification of the method of Tanzer and Gilvarg (18)

The author determined S-CPK in 120 patients suspected of coronary occlusion. S-CPK was determined for three or more days after the admission of the patients. The diagnosis of myocardial infarction was established in 64 patients. Of these patients, 57 exhibited increased values of S-CPK. In 11 patients with normal values of S-CPK the determination were performed more than 72 hours after the onset of the disease The last patient was examined 12 hours before the fatal infarction, at which time the S-CPK was found to be normal

It is found that the increase above normal limits of S-CPK begins at an early stage of the disease in most cases. One patient showed an increased value of S-CPK 3 hours after the onset of the disease. The elevation of S-CPK is of only short duration. 40 of the patients showed normal values on the third day of the disease

Elevation of the S-CPK on the third day of the disease seems to indicate a grave prognosis, as 40 of the patients with elevated values at this time died. In contrast with this, it was found that only 17 % of the patients with normal values of S-CPK on the third day of the disease died. 23 of the patients in the entire series with coronary occlusion died

Five patients exhibited elevated values of S-CPK. In these five cases it was impossible with certainty to establish a final diagnosis of myocardial infarction. On the basis of the case histories it is shown that all these patients might have suffered from this disease.

Twenty-seven patients with arteriosclerotic or hypertensive heart disease had S-CPK values within the normal range. Seven patients with various types of tachycardia exhibited normal values of S-CPK.

Furthermore, normal values of S-CPK were found in patients with various lung diseases, including infarction of the lung. Patients with various abdominal disorders (cholelithiasis, gastric ulcer) had normal values. Especially normal values of S-CPK was demonstrated in seven patients with damage of the liver parenchyma.

In the evaluation of the results of the determinations of S-CPK in the 120 individual cases the author arrived at the conclusion that the determination of S-CPK provided a definite additional aid in establishing the final diagnosis in 30 of the patients.

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From the Frederiksborg County Central Hospital, Medical Department F
(Head Mogens I ersen, M. D.) Hillerød, Denmark

Corticosteroid Osteoporosis and Treatment with Anabolic Hormone

By

HANS LUND

Osteoporosis is a common complication to treatment w th corticosteroids. It has been established also as one of the symptoms of endogenous hypercorticism in Cushing s disease in which the incidence has been reported to be as high as 88 and 83 per cent (3 7)

According to Albright (1) osteoporosis is a condition of bone atrophy caused by a relative imbalance between anabolic and catabolic hormones. In hypercorticism excessive upply of catabolic hormones brings about destruction of the protein matrix of the skeleton with a secondary loss of calcium salts. Anabolism is effected by the sex hormones. Albright (1) considers oestrogens to stimulate osteoblastic activity while androgens possibly cause anabolism either by suppressing the adrenals or by an effect directly on the cell protoplasm, or both.

The effect of anabolic steroids has since been demonstrated in several ex periments. Corticosteroid induced negative nitrogen- and calcium balances were reversed by arious anabolic hormones as regard the nitrogen balance whereas the calcium balance albeit less negative on the whole remained so under this treatment (11) In his balance study Sagild (9) in senile osteoporosis demonstrated a strong anabolic effect on nitrogen- as well as calcium-balance following admini tration of nortestosterone.

Although anabolic hormones when used in the treatment of osteoporosis in human beings appear to relieve the bone pains to some degree there is as yet little evidence that restitution of bone density ever occurs, despite those few cases reported by Sherman (10) Polshuk and and Kleinhause (8) and Cooke (2) all treated with oestrogens. A review by Henneman and Wallach (5) of 200 cases of senile osteoporosis treated with oestrogens revealed no demonstrabl radiographic improvement, even after prolonged periods of treatment of up to 20 years. This is a weakness of Albright s theory of osteoporosis mainly caused by a

Submitted for publication May 22, 1963

Five patients exhibited elevated values of S-CPK. In these five cases it was impossible with certainty to establish a final diagnosis of myocardial infarction. On the basis of the case histories it is shown that all these patients might have suffered from this disease.

Twenty-seven patients with arteriosclerotic or hypertensive heart disease had S-CPK values within the normal range. Seven patients with various types of tachycardia exhibited normal values of S-CPK

Furthermore, normal values of S-CPK were found in patients with various lung diseases, including infarction of the lung Patients with various abdominal disorders (cholelithiasis, gastric ulcer) had normal values. Especially normal values of S-CPK was demonstrated in seven patients with damage of the liver parenchyma

In the evaluation of the results of the determinations of S-CPK in the 120 individual cases the author arrived at the conclusion that the determination of S-CPK provided a definite additional aid in establishing the final diagnosis in 30 of the patients.

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Table I Survey of patients treated with corticosteroids, concerning diagnosis anabolic therapy and occurrence of osteoporosis

	Rheumatoid arthritis 21		Other diseases 50	
	Anabolic therapy 8	No anabolic therapy 13	Anabolic therapy 30	No anabolic therapy 20
Osteoporosis Total no. 41	7	11	15	8
No osteoporosis Total no. 30	1	2	15	12

Table II Survey of patients treated with corticosteroids, showing osteoporosis of the vertebral column in relation to sex age duration of corticosteroid treatment and anabolic therapy or not Number of cases showing fractures are recorded in parenthesis

		Rheumatoid arthritis 18 (3)	Other diseases 23 (4)	Total 41 (7)
Sex	♂	3	10 (3)	13 (3)
	♀	15 (3)	13 (1)	28 (4)
Age	< 50 yrs	3	0	3
	> 50 yrs	15 (3)	23 (4)	38 (7)
Duration of corticosteroid treatment	0.5—2 yrs	1	7	8
	> 2 yrs	17 (3)	16 (4)	33 (7)
Anabolic therapy	+	7 (2)	15 (4)	22 (6)
	0	11 (1)	8	19 (1)

deficiency of anabolic hormones, and has led to other hypotheses concerning the pathogenesis of osteoporosis.

Thus Nordin (6) suggests senile osteoporosis to be due to a long-continued negative calcium balance caused by a deficiency of dietary calcium. This was supported by Harrison et al. (4) who in their study demonstrated that many patients suffering from this form of osteoporosis absorb and retain calcium to an abnormal degree when on a high calcium intake, and moreover may continue to do so as the observations were carried out (3 1/2 years) There was also relief of the subjective symptoms although X rays did not show any increased density of the bones.

Considering the results of anabolic therapy in osteoporosis, it seems as if there is clear contrast to the promising effect obtained in the laboratory and the very few and scattered examples of radiographic remission mentioned in the literature.

This obvious discrepancy has led to the present study

Material and methods

The material consists of patients treated with corticosteroids for more than six months. Treatment with steroids was started in 1954 or later Until 1959 no anabolic therapy was given. Since then supplementary therapy with norandrosteronephenylpropionate (NAPP) Norabol® has been used as an experimental prophylaxis of osteoporosis. The patients treated with corticosteroids in the preprophylactic period serve as controls.

The material comprises 71 patients, 40 females, aged 60 years on an average and 31 males with an average age of 59 years.

Indications of steroid therapy have been manifold, bronchial asthma, rheumatoid arthritis, and collagen diseases being the prevalent groups. In a few instances steroid treatment has been given to patients suffering from cirrhosis of the liver nephrosis, dermatological, and eye diseases. An average corticosteroid dose has been prednisone 7 mg day

The dose of NAPP has been 25 mg i.m./2 weeks. A few of the patients developing severe osteoporotic changes, i.e. some of the reported fractures, have had their dose increased to 50 mg/week.

X-rays of the vertebral column have been taken regularly in most cases with an interval of 6 months, and studied by a specialist taking no interest in the present investigation.

The cases in which X-rays have shown osteoporosis from the beginning of steroid therapy as well as those giving information of adequate trauma of their backs have been excluded. It has also been noted that none of the patients suffered from hyperparathyroidism or neoplastic bone disease.

Results

In table I a survey of the complete material is given. Rheumatoid arthritis is recorded as a special group because of the frequency per se of osteoporosis in this disease.

Table II illustrates the patients showing osteoporosis. The cases of osteoporosis showing one or more fractures of the vertebrae are recorded in parenthesis.

Not recorded in the tables is a pelvic fracture occurring in one of the osteoporotic patients during treatment with NAPP However this patient, a woman suffering from rheumatoid arthritis might have had an adequate trauma, as during the treatment period she fell in the street.

Forty-one cases of osteoporosis have been discovered. Of these 7 had one or more fractures of the vertebrae. Twenty two i. e. a little more than half of the cases of osteoporosis, including 6 of the 7 fractures were discovered during prophylactic treatment with NAPP In none of the cases was a radiographic remission demonstrable.

Discussion and conclusion

The results seem to indicate very strongly that NAPP had no effect in the prophylaxis of corticosteroid osteoporosis, at least in the dosage given in this series.

Some might doubt the significance of the group of clean osteoporosis, i. e. the cases showing decreased density of the vertebrae without fracture, considering the difficulty in estimating a slight degree of osteoporosis on X ray However as previously emphasized, the X ray investigation has been performed by a neutral observer not by the author So it would be expected that the relative inaccuracy of his observations should influence the NAPP treated- and untreated groups equally and not disturb the final results as a whole As regards the fractures, however no such inaccuracy can be claimed and the number of these should be large enough to justify the above made conclusion

Summary

The literature on the treatment of osteoporosis with anabolic hormones is reviewed briefly

A report is given on the results of prophylactic treatment with norandrosteronephenylpropionate (NAPP) in a series of patients treated with corticosteroids.

No restitution of bone density has been obtained. Comparison with a control group having had no treatment with NAPP showed no significant difference in the treated- and untreated groups.

From this it is concluded that treatment with NAPP has had no effect in inhibiting corticosteroid osteoporosis.

Acknowledgment

I thank Torben Andersen, M. D. (Head of Medical Department B) for his permission to include patients from Medical Department B in the material.

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From the Medical Department A, The University Hospital, and The Institute of Medical Physiology University of Copenhagen, Denmark

Investigations into the Thiazide-induced Antidiuresis in Patients with Diabetes Insipidus

By

Erik Skadhauge

In 1959—60 an antidiuretic action of diuretics of the thiazide group in diabetes insipidus (d.i.) was described independently by various authors (10 28, 31)

The thiazides (tz.) may reduce the diuresis by 30—60 when given orally in the same doses as are used in the treatment of oedema, i.e. doses which have a pronounced natriuretic effect (2 10 11 12, 17 19 20 23 2․ 28, 31 32 37) The antidiuresis is reduced after a few weeks of treatment.

I the various reports concerning the tz.-induced antidiuresis, different possibilities for the mode of action have been discussed Some investigators report a reduction of the glomerular filtration rate (GFR (2 20, 25) others have not been able to confirm this finding (10 28, 31) A reduced GFR may lead to an increased renal concentration capacity In some reports a decrease of the plasma sodium and osmolality was found (2 28, 31 32 37) both in adult patients with vasopressin-sensitiv d.i. and in infants with nephrogenic d.i. This decrease may reduce the thirst.

In an attempt to help elucidate the mode of action the results of tz. treatment of 7 patients with d.i are reported in the present paper

Material and methods

Clinical

The patients were admitted t he metabolic ward of the medical department A (pat. no. 4 to the dept. of pediatrics The diagnoses of the patients wer as follow (duration of the polyuria in parentheses Pat. no 1 A 50-year-old female with idiopathic, vasopressin-sensitiv d.i. (30 yrs) Pa no. 2 A 21-year old male with vasopressin-sensitiv d.i. and Hand-Schuller-Christian disease (15 yrs) Pat. no. 3 A 22-year-old female with cryptogenic panhypopituitarism (13 yrs) Pat. no. 4 A 5-year-old male with vasopressin-sensitive d.i. and a previous head trauma (2 yrs) Pat no. 5 A 17-year-old male with vasopressin-sensitive d.i. and sarcoidosis (2 yrs) Pat. no. 6 A 26-year-old female with psychogenic polydipsia with complete suppression of the release of antidiuretic hormone as described by Decourt and Hurez (14) and Wedeen (38) (since birth?) Pat. no. 7 A 52-year-old female with vasopressin-sensitiv d.i. and sarcoidosis(?) (2 1/2 yrs)

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Table I Metabolic data for the 7 patients during the various periods of the study The average values in standard deviation (SD) for the whole period and the number of degrees of freedom (F) are given for plasma

Pat. no.	Treatment	Weight (kg)	Plasma				
			Na (mEq/l)	Cl (mEq/l)	Osm (mOsm/kg)	K (mEq/l)	HCO^- (mEq/l)
1	Vasopressin	63.0 (4)	137 (3)	100 (3)	278 (3)	4.0 (3)	24.5 (3)
	No treatment	61.2 (6)	145 (2)	107 (2)	296 (3)	3.4 (2)	28.3 (2)
	Thiazide	60.3 (7)	139 (4)	99 (4)	262 (2)	3.1 (6)	27.8 (3)
	SD/F		2.2/6	3.0/6	12 6/5		
2	Vasopressin	58.9 (4)	138 (2)	104 (2)	289 (2)	3.9 (2)	22.0 (2)
	No treatment	57.6 (5)	147 (4)	108 (4)	302 (4)	4.3 (4)	23.2 (4)
	Thiazide	57 4 (5)	143 (4)	99 (4)	290 (4)	3.5 (4)	26.8 (4)
	SD/F		0.9/7	1.9/7	3.3/7		
3	Vasopressin	55.4 (4)	131 (3)	95 (3)	264 (3)	4.6 (3)	22.6 (3)
	No treatment	54.2 (4)	139 (3)	103 (3)	323 (2)	4.8 (3)	23.0 (3)
	Thiazide	53.1 (4)	138 (4)	94 (4)	275 (4)	3.4 (4)	27.5 (4)
	SD/F		2.4/7	4 0/7	10.6/6		
4	Vasopressin	26.9 (2)	141 (2)	100 (2)	283 (1)	4 6 (2)	21.8 (1)
	No treatment	26.9 (5)	142 (4)	103 (3)	291 (2)	4.2 (4)	24.3 (3)
	Thiazide	25.8 (4)	139 (3)	96 (2)	290 (2)	3.4 (3)	26.4 (3)
	SD/F		1.3/6	3.0/4	2.1/2		
5	No treatment	70.5 (5)	142 (3)	100 (3)	292 (2)	4.2 (3)	23.6 (3)
	Thiazide	69 6 (3)	143 (3)	96 (3)	285 (1)	4.2 (2)	25.1 (2)
	T + spirolact.	68.5 (3)	141 (3)	96 (3)	281 (3)	3.7 (3)	24.3 (3)
	Salt-f. diet	68.6 (6)	144 (1)	103 (1)	290 (1)	4.1 (1)	25.8 (1)
	Salt f. diet + T	68.0 (1)	142 (2)	102 (2)	290 (2)	4 1 (2)	25.3 (2)
	SD/F		2 7/9	0.8/3	3.0/6		
6	No treatment	54 8 (11)	136 (5)	103 (4)	280 (1)	3.7 (5)	22.6 (5)
	Salt-f. diet	54.3 (3)	135 (1)	104 (1)	280 (1)	3.4 (1)	23.1 (1)
	House-diet + T	53.1 (4)	132 (2)	93 (2)	269 (2)	3.0 (2)	24.9 (2)
	SD/F		2.1/5	3.6/4	1 4/1		
7	Thiazid	72.1 (4)	143 (1)	—	—	4.7 (1)	25.0 (1)
	No treatment	69.2 (4)	150 (2)	—	320 (3)	4 4 (2)	—
	Thiazide	68.5 (4)	139 (3)	—	281 (3)	3.3 (4)	24.5 (1)
	SD/F		2.1/3		3.7/4		

The diagnoses were confirmed by the use of a water deprivation test, a NaCl infusion test, and a vasopressin test

The various periods of treatment for each patient are indicated in *table I* Patients no. 1 2 and 4 received vasopressin treatment prior to admission. This medication was discontinued after 4—6 days, and after a week without treatment tx. was given for 6—7 days. Patients no. 3 5 and 6 were untreated at

the different treatments and the number of observations (in parentheses) are recorded. For each patient the sodium, [illegible] serum chloride and creatinine clearance

Urine		Clearance		Uric acid (mg %)	C_{H_2O} (ml)	Cosm (ml)
Diuresis (ml/24 hrs)	Osm (mOsm/kg)	Creat. (ml/min)	Urea (ml/min)			
942 (5)	622 (5)	91 (5)	—	4.8	—	—
3,824 (6)	115 (5)	88 (6)	43 (5)	—	3,772 (5)	2,236 (6)
3,011 (6)	198 (6)	80 (7)	40 (8)	5.3	833 (6)	2,160 (6)
		7.6/17				
1,067 (5)	570 (3)	85 (3)	—	—	—	—
6,275 (4)	81 (3)	101 (3)	—	—	4,845 (3)	1,763 (3)
4,525 (5)	185 (5)	99 (6)	—	7.6	1,595 (5)	2,928 (5)
		30.2/11				
785 (4)	445 (4)	51 (3)	—	4.5	—	—
4,245 (4)	90 (4)	45 (4)	—	—	3,065 (4)	1,180 (4)
2,569 (4)	108 (4)	37 (5)	—	—	1,570 (4)	989 (4)
		12.5/11				
1,324 (5)	467 (5)	64 (3)	48 (4)	—	—	—
7,131 (4)	73 (3)	51 (3)	44 (5)	—	5,195 (3)	1,747 (3)
4,720 (5)	164 (5)	53 (6)	48 (6)	—	3,010 (5)	1,696 (5)
		13.[illegible]/11				
8,232 (5)	105 (5)	114 (5)	—	—	5,272 (5)	2,960 (5)
5,398 (3)	114 (3)	94 (4)	—	—	3,220 (3)	2,1[illegible] (3)
4,956 (3)	141 (2)	81 (6)	—	7.4	2,068 (2)	2,098 (2)
6,167 (6)	60 (6)	97 (6)	—	—	—	—
4,110 (1)	101 (1)	98 (2)	—	—	—	—
		13.2/22				
6,171 (11)	128 (11)	133 (5)	—	4.6	—	—
4,313 (5)	129 (3)	131 (4)	—	—	2,343 (3)	1,970 (3)
1,673 (3)	333 (3)	123 (4)	—	6.1	−503 (3)	2,177 (3)
		22.2/12				
1,902 (11)	—	58 (1)	—	—	—	—
3,223 (4)	128 (4)	64 (3)	—	—	1,935 (3)	1,290 (3)
2,106 (5)	244 (5)	35 (3)	—	5.7–5.5	501 (5)	1,803 (3)
		3.6/8				

admission. Patient no. 3 received no treatment during the first week, thereafter vasopressin for 6 days, and later tz. treatment. Patient no. 5 received no treatment for 5 days, then tz. + spirolactone during 7 days, followed by 6 days without treatment. After an intercurren period with Hg-diuretic treatment the patient was given salt-free diet (10 mEq Na/24 hrs) for 12 days, combined with tz. during the last 3 days of that period. Pa-

Table I Metabolic data for the 7 patients during the various periods of the study The average values ... standard deviation (SD) for the whole period and the number of degrees of freedom (F) are given for plasma

Pat. no	Treatment	Weight (kg)	Plasma Na (mEq/l)	Cl (mEq/l)	Osm (mOsm/kg)	K (mEq/l)	HCO^- (mEq/l)
1	Vasopressin	63.0 (4)	137 (3)	100 (3)	278 (3)	4.0 (3)	24.5 (3)
	No treatment	61.2 (6)	145 (2)	107 (2)	296 (3)	3.4 (2)	28.3 (2)
	Thiazide	60.3 (7)	139 (4)	99 (4)	262 (2)	3.1 (6)	27.8 (3)
	SD/F		2.2/6	3.0/6	12.6/5		
2	Vasopressin	58.9 (4)	138 (2)	104 (2)	289 (2)	3.9 (2)	22.0 (2)
	No treatment	57.6 (3)	147 (4)	108 (4)	302 (4)	4.3 (4)	23.2 (4)
	Thiazide	57.4 (5)	143 (4)	99 (4)	290 (4)	3.5 (4)	26.8 (4)
	SD/F		0.9/7	1.9/7	3.3/7		
3	Vasopressin	55.4 (4)	131 (3)	95 (3)	264 (3)	4.6 (3)	22.6 (3)
	No treatment	54.2 (4)	139 (3)	103 (3)	323 (2)	4.8 (3)	23.0 (3)
	Thiazide	53.1 (4)	138 (4)	94 (4)	275 (4)	3.4 (4)	27.5 (4)
	SD/F		2.4/7	4.0/7	10.6/6		
4	Vasopressin	26.9 (2)	141 (2)	100 (2)	285 (1)	4.6 (2)	21.8 (1)
	No treatment	26.9 (5)	142 (4)	103 (3)	291 (2)	4.2 (4)	24.3 (3)
	Thiazide	25.8 (4)	139 (3)	96 (2)	290 (2)	3.4 (3)	26.4 (3)
	SD/F		1.3/6	3.0/4	2.1/2		
5	No treatment	70.5 (5)	142 (3)	100 (3)	292 (2)	4.2 (3)	23.6 (3)
	Thiazide	69.6 (3)	143 (3)	96 (3)	285 (1)	4.2 (2)	25.1 (2)
	T + spirolact.	68.5 (3)	141 (3)	96 (3)	281 (3)	3.7 (3)	24.3 (3)
	Salt-f. diet	68.6 (6)	144 (1)	103 (1)	290 (1)	4.1 (1)	25.8 (1)
	Salt-f. diet + T	68.0 (1)	142 (2)	102 (2)	290 (2)	4.1 (2)	25.3 (2)
	SD/F		2.7/9	0.8/3	3.0/6		
6	No treatment	54.8 (11)	136 (5)	103 (4)	280 (1)	3.7 (5)	22.6 (5)
	Salt-f. diet	54.3 (3)	135 (1)	104 (1)	280 (1)	3.4 (1)	23.1 (1)
	House-diet + T	53.1 (4)	132 (2)	93 (2)	269 (2)	3.0 (2)	24.9 (2)
	SD/F		2.1/5	3.6/4	1.4/1		
7	Thiazide	72.1 (4)	143 (1)	—	—	4.7 (1)	25.0 (1)
	No treatment	69.2 (4)	150 (2)	—	320 (3)	4.4 (2)	—
	Thiazide	68.5 (4)	139 (3)	—	281 (3)	3.3 (4)	24.5 (1)
	SD/F		2.1/3		5.7/4		

The diagnoses were confirmed by the use of a water deprivation test, a NaCl-infusion test, and a vasopressin test.

The various periods of treatment for each patient are indicated in table I. Patients no. 1, 2, and 4 received vasopressin treatment prior to admission. This medication was discontinued after 4—6 days, and after a week without treatment tz. was given for 6—7 days. Patients no. 3, 5 and 6 were untreated at

the different treatments and the number of observations (in parentheses) are recorded. For each patient the sodium, osmolality, serum chloride and creatinine clearance

Urine		Clearance		Uric acid (mg %)	C_{H_2O} (ml)	Cosm (ml)
Diuresis (ml/24 hrs)	Osm (mOsm/kg)	Creat. (ml/min)	Urea (ml/min)			
942 (3)	622 (5)	91 (5)	—	4.8	—	—
5,824 (6)	115 (3)	88 (6)	43 (3)	—	3,772 (5)	2,236 (6)
3,011 (6)	198 (6)	89 (7)	40 (8)	5.3	853 (6)	2,160 (6)
		7.6/17				
1,067 (3)	70 (3)	83 (3)	—	—	—	—
6,275 (4)	81 (3)	101 (3)	—	—	4,845 (3)	1 765 (3)
4,323 (5)	185 (5)	99 (6)	—	7.6	1,595 (3)	2,928 (5)
		30.2/11				
783 (4)	445 (4)	51 (5)	—	4.5	—	—
4245 (4)	90 (4)	45 (4)	—	—	3 065 (4)	1 180 (4)
2,359 (4)	108 (4)	37 (5)	—	—	1,570 (4)	889 (4)
		12.9 11				
1,514 (5)	467 (5)	64 (5)	48 (4)	—		—
7,131 (4)	73 (5)	51 (5)	44 (5)	—	5 195 (3)	1 747 (3)
4,726 (5)	104 (5)	53 (6)	48 (6)	—	3,010 (5)	1 696 (5)
		13.2/11				
8,232 (3)	103 (5)	114 (5)	—	—	5,272 (5)	2,960 (5)
3,598 (3)	114 (3)	91 (4)	—	—	3,220 (3)	2,177 (3)
4,056 (3)	141 (2)	81 (6)	—	7.4	2,068 (2)	2,098 (2)
6 167 (6)	60 (6)	97 (6)	—	—	—	—
4,110 (1)	101 (1)	98 (2)	—	—	—	—
		13.2/22				
6,171 (11)	126 (11)	153 (5)	—	4.6	—	—
4,313 (3)	129 (3)	131 (4)	—	—	2,343 (3)	1,970 (3)
1,673 (3)	333 (3)	125 (4)	—	6.1	−503 (3)	2,177 (3)
		22.2/12				
1,902 (11)	—	56 (1)	—	—	—	—
3,223 (4)	126 (4)	64 (3)	—	—	1,933 (3)	1,290 (3)
2,106 (5)	244 (5)	55 (5)	—	5.7 – 5.5	301 (5)	1,805 (5)
		5.6/8				

admission. Patient no. 3 received no treatment during the first week, thereafter vasopressin for 6 days, and later tz. treatment. Patient no. 5 received no treatment for 5 days, then tz. + spirolactone during 7 days, followed by 6 days without treatment. After an intercurrent period with Hg-diuretic treatment the patient was given salt-free diet (10 mEq Na/24 hrs) for 12 days, combined with tz. during the last 3 days of that period. Pa-

Table I Metabolic data for the 7 patients during the various periods of the study The average values ± standard deviation (SD) for the whole period and the number of degrees of freedom (F) are given for plasma

Pat. no	Treatment	Weight (kg)	Plasma Na (mEq/l)	Cl (mEq/l)	Osm (mOsm/kg)	K (mEq/l)	HCO^- (mEq/l)
1	Vasopressin	63.0 (4)	137 (3)	100 (3)	278 (3)	4.0 (3)	24.5 (3)
	No treatment	61.2 (6)	145 (2)	107 (2)	296 (3)	3.4 (*)	28.3 (2)
	Thiazide	60.3 (7)	139 (4)	99 (4)	262 (2)	3.1 (6)	27.8 (3)
	SD/F		2.2/6	3.0/6	12.6/5		
2	Vasopressin	58.9 (4)	138 (2)	104 (2)	289 (2)	3.9 (2)	22.0 (2)
	No treatment	57.6 (5)	147 (4)	108 (4)	302 (4)	4.3 (4)	23.2 (4)
	Thiazide	57.4 (5)	143 (4)	99 (4)	290 (4)	3.5 (4)	26.8 (4)
	SD/F		0.9/7	1.9/7	3.3/7		
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	SD/F		2.4/7	4.0/7	10.6/6		
4	Vasopressin	26.9 (2)	141 (2)	100 (2)	285 (1)	4.6 (*)	21.3 (1)
	No treatment	26.9 (5)	142 (4)	103 (3)	291 (2)	4.2 (4)	24.3 (3)
	Thiazide	25.8 (4)	139 (3)	96 (2)	290 (2)	3.4 (3)	26.4 (3)
	SD/F		1.3/6	3.0/4	2.1/2		
5	No treatment	70.5 (5)	142 (3)	100 (3)	292 (2)	4.2 (3)	23.6 (3)
	Thiazid	69.6 (3)	143 (3)	96 (3)	285 (1)	4.2 (2)	25.1 (2)
	T + spirolact.	68.5 (3)	141 (3)	96 (3)	281 (3)	3.7 (3)	24.3 (3)
	Salt-f. diet	68.6 (6)	144 (1)	103 (1)	290 (1)	4.1 (1)	23.8 (1)
	Salt f. diet + T	68.0 (1)	142 (2)	102 (2)	290 (2)	4.1 (2)	23.3 (2)
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	SD/F		2.1/5	3.6/4	1.4/1		
7	Thiazide	72.1 (4)	143 (1)	—	—	4.7 (1)	25.0 (1)
	No treatment	69.2 (4)	150 (2)	—	320 (3)	4.4 (*)	—
	Thiazide	68.5 (4)	139 (3)	—	281 (3)	3.3 (4)	24.5 (1)
	SD/F		2.1/3		5.7/4		

The diagnoses were confirmed by the use of a water deprivation test, a NaCl-infusion test, and a vasopressin test.

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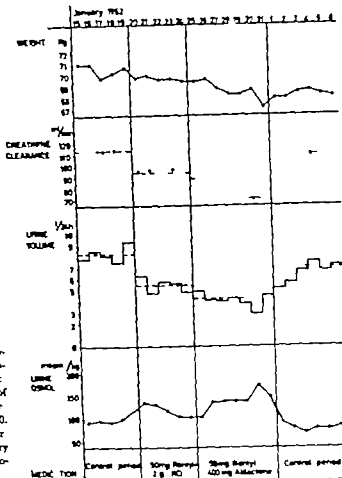


Fig. 2. Effects of thiazide (Rontyl®), and thiazide in combination with spirolactone (Aldactone®). A significant reduction of the GFR is seen during both periods of treatment. On Jan. 30, water deprivation test was carried out, and there ensued very low diuresis and high urine osmolality

response is shown. The diuresis is gradually reduced to a minimum on the third to fourth day. After discontinuation of the tz. treatment the diuresis gradually increases, and generally it reaches the untreated level on the third to fourth day. In patient 5 slightly lower level is reached (fig. 2). A weight loss of approximately one kg is seen both after discontinuation of vasopressin and following tz. administration.

Urine osmolality

The increase of the urine osmolality varies greatly 7—128 %, from 98 to 159 mOsm/kg on the average (fig. 3). The increase is greatest in the first days of the tz. medication period. The natriuresis follows the generally observed pattern for the action of tz. Initially a considerable natriuresis is seen, which decreases during the third to fourth day (fig. 4). A pronounced kaliuresis is seen. This makes necessary a large increase in potassium intake. The chloriuresis is also increased.

The free water clearance was calculated from the formula $C_{H_2O} = V - \frac{U_{osm} \cdot V}{P_{osm}}$ (V = volume of urine. Uosm and Posm = urine and plasma osmolality) (table

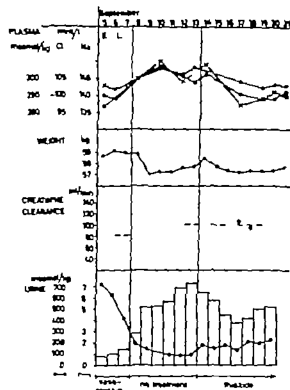


Fig 1 Plasma and urine values, together with the weight and the creatinine clearance for patient no. 2 during the 3 periods of the metabolic study The clearances varied considerably in spite of fairly constant day to-day amounts of creatinine in the urine.

tient no 6 Normal diet (6 days) salt free diet (5 days) salt-free diet + tz. (3 days) and normal diet + tz. (2 days) Patient no. 7 received tz. prior to admission. This treatment was discontinued for two weeks, and thereafter tz. was given for one week.

Regimen

All the subjects received a diet with a constant daily content of protein, sodium, and potassium composed from tables the average daily intakes were protein 50 g sodium 80 mEq and potassium 60 mEq/24 hrs. Water intake and diuresis were measured daily and the patients were weighed each morning after voiding and before breakfast. 24-hour urine specimens were analysed for sodium, potassium, creatinine and osmolality In addition urinary urea (pat. 1 and 4) and chloride (pat. 2 3 4 and 6) were determined Plasma sodium, potassium, osmolality serum chloride and creatinine, standard HCO3, and urea (pat. 1 and 4) were determined three times a week.

Drugs

Thiazide Rontyl® (hydroflumethiazide) 50—75 mg daily with KCl enterosoluble 2—5 g (pat 4 Centyl® (bendroflumethiazide) 5—7.5 mg) Spirolactone Aldactone® 25 mg t. i. d Vasopressin Insipidin Retard® a commercial extract of hog posterior pituitaries, with delayed action due to combination with polyvinylpyrrolidone, 0.3—1 0 ml daily (1 ml = 20 I U)

Chemical analysis

Urine and plasma osmolalities were determined on 2 ml samples with a commercial electric freezing point thermometer (Fiske osmometer) All other analyses have been carried out as routine analyses at the department of Clinical Chemistry The University Hospital.

Results

The results are recorded in table I Only values from the third day following any change of treatment are included as any new level of the blood and urine values would have been established by this time. However any change on the second day had the same direction as the established level. Thus an average decrease in body weight of 0 7 kg, plasma osmolality of 15 mOsm/kg and serum chloride of 6 mEq/l was observed The values for creatinine and urea clearance on tz. treatment are included from the second day On the days when no venipuncture has been performed values for serum creatinine urea and plasma osmolality to be used in the calculation of clearances have been obtained by interpolation

Reduction of diuresis

The urine volume is reduced by 28—39 on the average to 2 3 (from 5 822 to 3 720 l/24 hrs) In fig 1 a typical

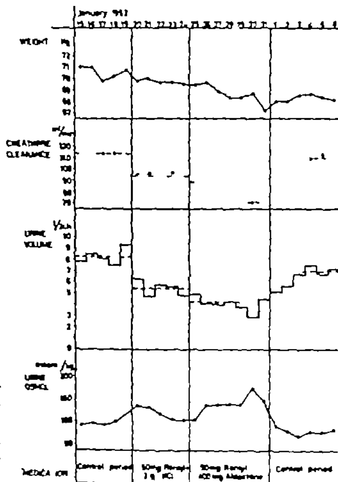


Fig. 2. Effects of thiazide (Rontyl), and thiazide in combination with spirolactone (Aldactone®). A significant reduction of the GFR is seen during both periods of treatment. On Jan. 30 water deprivation test was carried out, and there ensued a very low diuresis and high urine osmolality

response is shown. The diuresis is gradually reduced to a minimum on the third to fourth day. After discontinuation of the tz. treatment the diuresis gradually increases, and generally it reaches the untreated level on the third to fourth day. In patient 5 a slightly lower level is reached (fig. 2). A weight loss of approximately one kg is seen both after discontinuation of vasopressin and following tz. administration.

Urine osmolality

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The free water clearance was calculated from the formula $C_{H_2O} = V - \frac{Uosm.V}{Posm}$ (V = volume of urine, Uosm and Posm = urine and plasma osmolality) (table

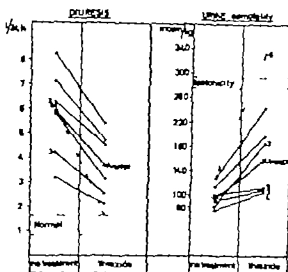


Fig 3 The antidiuresis, i.e. the reduction of the diuresis with the concomitant increase of the osmolality A normal diuresis and tonicity is reached in patient 6. This is interpreted partly as a placebo reaction, since the patient had psychogenic polydipsia (p.p.)

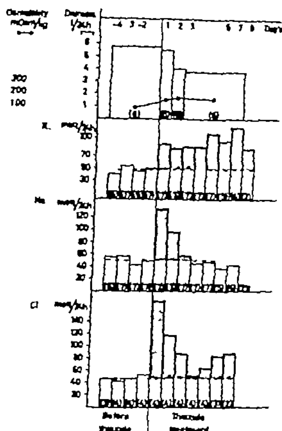


Fig 4 Average values for the antidiuresis and the increase of the electrolyte excretion initiated by thiazide The figures in parentheses indicate the number of patients.

I) The C_{H_2O} falls during tz therapy as expected. It is however clear that the C_{H_2O} expresses the antidiuretic effect rather poorly when the tz. induced natriuresis is pronounced since the latter will change the diuresis and urine osmolality Even if it is assumed that the tz. induced increase of the urinary sodium content is due mainly to a decrease of the isotonic proximal tubular reabsorption measurement of C_{H_2O} fails to recognize *changes in the concentration capacity of* the distal tubular system

Instead the "water loss" will be expressed here as the number of liters of urine which, with the actual osmolality *will excrete 1 osmol* Before the calculation of this value the diuresis has been corrected for the part of the diuresis which "contains" the extra equivalents of electrolytes, $Na^+ + K^+ + Cl^-$ which are excreted due to the saluretic action of tz. with the assumption of a coefficient of activity of one Thus, the "antidiuresis" *in this definition is based on the final* composition of the urine.

These calculations have been made for patients 2 3 and 4 (fig 5) The diuresis is shown (fig 5 III) with and without correction for the extra diuresis (V_{el}) which "contains" the extra equivalents of electrolytes (Na+K+Cl) excreted $V_{el} = \left(\frac{Na+K+Cl}{Uosm}\right)$ The "antidiuresis" is taken to be the decrease in "water loss" measured as the diuresis per osmol with and without correction for V_{el} When these corrections are made (changes in plasma osmolality were neglected) it will be seen that the antidiuresis reaches the

full degree even on the first day of treatment.

The patients had a decreased feeling of thirst from the first day of treatment (placebo tests were not made)

The patient with psychogenic polydipsia, no. 6 had a hypertonic urine on tz. treatment (fig 3) This is considered partly to be a placebo effect, since tz. has never been able to make the urine hypertonic when given to severely polyuric rats (23) and — even in high doses — has not been able to reduce the diuresis below 50 (11)

Patients no. 1 2, and 7 show a larger increase in urine osmolality than patients no. 3 4 and 5 This difference is concomitant with a difference in kaliuresis Pat. 1 2, and 7 Na 55 K 119 mEq/24 hrs, Pat. 3 4 and 5 Na 47 K 60 mEq/24 hrs (average values for the 3rd—6th day of therapy)

Plasma osmolality and electrolyte concentrations

After discontinuation of vasopressin an increase of plasma sodium, osmolality and serum chloride was constantly seen — these values were reduced on tz. treatment. The average fall was sodium 4 mEq/l, chloride 8 mEq/l, osmolality 21 mOsm/kg In some of the patients the plasma osmolality (pat. 1 2, 3 and 7) and sodium (pat. 2 and 7) exceeded the normal range in the untreated state Normal range plasma sodium 136—47 (2 S.D.) mEq/l. Plasma osmolality 280—94 mOsm/kg

Statistical significance of changes The standard deviations and number of degrees of freedom are given for the individual patients in table 1. Common variances have been calculated for sodium and for chloride respectively Na 4 4 Cl 8.6. The variance for the plasma

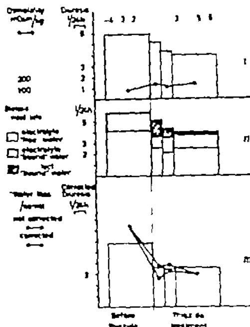


Fig 3 Average values for patients no. 2, 3, and 4 are shown. I shows diuresis and osmolality I II the diuresis has been divided into an electrolyte-containing and an electrolyte-free fraction on the basis of the actual osmolality Following the thiazide medication the extra excretion of electrolytes is separately shown. III demonstrates, as indicated in the text, that the reduction of the diuresis reaches its maximum even on the first day

osmolality (and creatinine clearance) deviates too much between the patients however a common variance for each patient throughout the treatment has been calculated. The significance has been tested by comparing the variance for the single patients and treatments with the common variance by a χ^2 distribution.

Plasma sodium was increased highly significantly ($P < 0.001$) after discontinuation of vasopressin in 3 patients out of 4 After tz. medication a highly significant decrease of the plasma sodium is seen in one patient, significant ($P < 0.01$) in two patients, and less significant

($P < 0.05$) in one patient out of seven. The increase of serum chloride after discontinuation of vasopressin was highly significant in two patients, less significant in one. After tz. medication the decrease was highly significant in three patients, significant in one, and less significant in one patient. On tz. medication the plasma osmolalities were reduced significantly in three patients and less significantly in one patient, whereas in three patients significance was not reached.

The glomerular filtration rate (GFR)

The GFR has been measured as the uncorrected endogenous 24-hour creatinine clearance for two of the patients in addition by urea clearance (no. 1 and 4). A significant reduction during tz. treatment was found only in the patient with the highest diuresis in the untreated state (no. 5, fig. 2, $P < 0.001$).

A salt free diet was followed by a reduction of the diuresis (pat. 5 and 6). When tz. was given, the antidiuresis was less pronounced than the reduction seen on a normal diet (pat. 5).

In patient 5 the following investigations were carried out. *Tz. + spirolactone.* This combined treatment was followed by a greater reduction of the urine volume and a greater urine osmolality (fig. 2). The spirolactone increased the natriuresis. After a *water deprivation test* to a weight loss of 1.2 kg on tz. + spirolactone treatment a pronounced antidiuresis was unexpectedly seen. There was a reduction of the diuresis to 30 ml/hr and an increase in urine osmolality to a maximum of 495 mOsm/kg. The day after this test was carried out the patient showed no antidiuresis in an i.v. NaCl-test. Before the onset of tz. therapy the water deprivation test to a weight loss of 1.7 kg showed a maximal urine osmolality of 114 mOsm/kg and a minimum diuresis of 260 ml/hr. With an interval of 5 days a Hg-diuretic (1 ml Thiomerin®) was given twice. The first injection was followed by an antidiuresis of two days duration with a maximal reduction of urine volume to 50 % concomitant with a pronounced natriuresis. After the second injection neither an antidiuresis nor a natriuresis ensued.

Continued treatment was given to patients 1, 3, 5 and 7. Patient 1 required a supply of vasopressin of approximately 1/4 of her previous dose after 3 weeks of tz. treatment. In patients 3 and 5 the antidiuretic effect continued practically unchanged after an observation period of 4 and 11 months respectively. Patients 2 and 4 felt at once that the antidiuretic effect was unsatisfactory. Patient 7 was well adapted to tz. at admission. Patient 6 did not want any treatment at all.

During the continued treatment the values for plasma sodium, osmolality and serum chloride remained within the normal range. The serum creatinine was normal. The initially increased values for standard HCO^- and uric acid were gradually normalized.

Side effects. The well known effect of the tz. — a hypokaliemic alkalosis — was observed. In spite of the potassium chloride supplement a clear but not significant fall of the plasma potassium (on the average from 4.1 to 3.4 mEq/l) was found concomitant with the increase of the standard HCO^-. A slight increase of the uric acid in serum was found. No other side effects were encountered.

Discussion

The antidiuretic effect of tz. in d.i. has been confirmed in the patients studied. The degree of antidiuresis is smaller than found by some of the previous authors.

It is, however impossible to compare directly since the antidiuresis depends not only on the tz. dose and the sodium and protein content of the diet, but perhaps also on the diuresis in the untreated state.

A significant reduction of plasma sodium and osmolality and of serum chloride, was found. In some of the patients the values for plasma sodium and osmolality were high in the untreated state. It is to be expected that a reduction after tz. treatment, as found here may reduce the thirst, since the threshold of thirst has been found to be an only 1—3 % reduction of the osmolality (3 40)

If the antidiuretic hormone has a direct thirst-reducing effect in patients and animals with d.i. (1 33) and if an increased sodium retention exists in untreated d.i., the thirst might be considered to create a polydipsia greater than warranted by the polyuria. If so tz may directly decrease the thirst. It has been found that the water intake may be reduced to a certain extent in untreated d.i. without weight loss or hemoconcentration both clinically (24) and experimentally (7 26) These last mentioned stud es are, however not conclusive, since the existence of a surgically produced, hypothalamic primary polydipsia has been demonstrated (5 35) The patients in this study felt the thirst decrease from the first day of treatment, and as calculated here, the antidiuresis is in full operation even from the first day In accordance with this a weight loss of one kg was seen

The quantity of the antidiuresis accords well with the maximal reduction of the diuresis that is seen on salt restricted diet (39) or following adrenalectomy of d.i. animals (23) In accordance with this smaller antidiuresis was found when tz. was given to one patient on a salt-free diet (pat. 5)

If a salt-free diet is given when the tz. treatment is discontinued, the diuresis will remain reduced (12 17) If an amount of sodium equivalent to the increase of the renal sodium excretion is given orally (12 20) no reduction of the diuresis is seen The urine osmolality must, however be increased. After adrenalectomy of d.i. rats only a slight antidiuresis is seen on tz. medication (23)

It has been observed that a state of sodium retention exists in d.i. patients and animals (18, 34) Increased values of plasma sodium and osmolality (5) have been found in the untreated state, when also the extracellular fluid volume (vasopressin-sensitive d.i.) (18, 27 29) and the total body water (unpublished observation) are high.

It may be concluded that tz., as a direct result of the natriuresis, can initiate a reduction of the d uresis, partly through a decrease of the thirst This concept is further confirmed by the finding of an antidiuresis after Hg-diuretics, as previously described (6 13 28) When Hg diuretics have been given to patients with d.i. (21) and to d.i. dogs (30) in maintained water diuresis, no antidiuresis has been observed. As the role of the polydipsia cannot be judged in these experiments they carry no weight in this connection.

A reduction of the thirst mechanism secondary to the natriuresis is, however clearly unsatisfactory as the only explanation of the tz. antidiuresis. It has been shown in rat experiments that a reduction of the allowed water intake to as low a level as is possible during tz. medication will lead to dehydration and death of the animals if instituted in the untreated state (23)

A renal component of the tz. antidiuresis has been observed previously In the

present study a statistically *significant* reduction of the GFR was found only in the patient who had the highest diuresis in the untreated state. In accordance with this, experiments with maintained water *diuresis have shown that tz. reduces the* GFR (22) and the reduction is greater when the level of hydration is higher (9) The renal action depends further on the tz. dose (8) The creatinine analyses, especially in serum, are rather inaccurate even when they are carried out correctly (16) Even the inulin-clearance determinations vary from day to day Accordingly the results of GFR determinations before and after tz. treatment have varied considerably However the GFR reducing ability of the tz. derivatives in states with a high degree of hydration both in d.i. and in hydrated normal subjects, seems well documented. A reduced GFR may in itself increase the renal concentration capacity to hypertonicity even when ADH is absent (36)

One patient was given a submaximal dose of tz. in combination with spirolactone. An increased natriuresis and antidiuresis was seen *If* spirolactone is given alone, a slight antidiuresis is seen (23 28) This indicates that the antidiuresis is secondary to the natriuresis, whether this is initiated mainly by an inhibition of the proximal or of the distal tubular reabsorption of sodium. An increased sodium reabsorption due to increased aldosterone level does not seem to be of importance for the antidiuresis.

The tz. induced natriuresis is considered mainly to be due to a reduced reabsorption of sodium in the proximal tubules the distal reabsorption is reduced only on higher tz. doses (8) The high urine osmolality reached *in one patient* in the water deprivation test on tz. + spirolactone (495 mOsm/kg) may be *due to an increased renal* papillary hypertonicity provided that no ADH was released on this occasion This concept is supported by the finding of an increased minimal urine osmolality on tz. treatment (19) and an increased maximal concentration capacity when vasopressin was given to d.i. patients after tz therapy (10) Direct measurement of the papillary hypertonicity in rats with experimental d.i. has, however not supported this hypothesis (4)

A direct permeability-increasing effect of the tz. on toad bladders has been described (15) but has not been confirmed by other workers (17) The transport of sodium ions has, however not been followed

It has been concluded by many authors that tz. may interfere with the sodium reabsorption at renal sites where the urine normally is diluted. This may lead to an increased osmolality but cannot in itself explain a reduced urine volume.

Summary

Plasma sodium osmolality and serum chloride, together with creatinine clearance, were studied before and during thiazide treatment of 7 patients with diabetes insipidus.

The diuresis was reduced by 28—39 % The urine osmolality was increased by 7—128 % An average decrease in plasma sodium of 4 mEq/l in plasma osmolality of 21 mOsm/kg, and in serum chloride of 8 mEq/l was observed Only in one patient was the creatinine clearance reduced significantly The thirst feeling was reduced even from the first day of treatment. The diuresis was not minimal until the third to fourth day If the diuresis was corrected for the extra electrolyte excretion initiated by the thiazide the antidiuresis was found to be operating to

the full extent even on the first day of treatment.

It is concluded that the antidiuresis is mainly secondary to the natriuresis and to a certain degree due to a decreased thirst feeling but there seems also to be a direct renal action, involving at least a reduction of the glomerular filtration rate. A pronounced antidiuresis was found by a water deprivation test after thiazide treatment in one patient.

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From the Department of Medicine (Head: E. Ask-Upmark, M. D.)
Royal University of Upsala, Sweden

The Laterality of the Carotid Murmur in Aortic Valvular Stenosis

By

E. Ask-Upmark and I. Cullhed

In aortic valvular stenosis the familiar systolic murmur is projected from its maximum beside the right border of the sternum at the level of the 2nd rib into the large arteries from the aortic arch. It may be listened to in the supraclavicular region (subclavian systems) or in the fossa carotica (carotid arteries). For several years it has been our impression that the murmur generally is more pronounced in the left than in the right carotid artery.

During the last 5 years 33 instances of verified aortic valvular stenosis have been recorded in our clinic. The diagnosis has been substantiated in all instances either by cardioangiography, by surgical intervention or and by necropsy. The cardioangiographies have been performed by our department of diagnostic roentgenology (head: Professor F. Knutsson, M. D.) in close collaboration with the cardiac laboratory of our clinic and with the department of physiology (head: Professor G. Ström, M. D.). The surgical operations have been carried out in our department of thoracic surgery (head: Professor V. O. Björk, M. D.) and the necropsies in our department of pathology (heads: Professor G. Hultquist, M. D. and Professor B. Engfelt, M. D.). The material is to be dealt with in detail by I. Cullhed in a thesis due to appear this year. In 2 instances there was an aortic coarctation as well (which had to be operated upon as a first step). In 7 instances there was also some degree of aortic insufficiency and in one case (a woman aged 26) there was a mitral stenosis, otherwise the aortic stenosis was the only valvular lesion encountered.

The distribution according to sex and age will be seen from table I.

As in other similar materials the male preponderance was considerable.

If this material is scrutinized as to the projection of the systolic murmur into the carotid arteries such a projection has,

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Table I

Age	Males	Females	All
15– 0	8	–	8
21–30	2	1	3
31–40	4	–	4
41–50	7	3	10
51–60	4	1	5
61–66	3	–	3
In all	28	5	33

of course, been found in all 33 instances. However, in only 11 has the laterality been recorded.

Among these 11 cases, in one the murmur was considered identical on both sides, in three it was more pronounced on the right side and in seven it was louder on the left side. Although limited the material available tends to confirm our initial impression. We may add that in most of the 22 cases without indicated laterality the murmur was really most pronounced at the left side although this fact has not been put in the records.

This observation may appear rather trivial. However it ought to be remembered that thrombosis of the internal carotid artery is met with much more commonly on the left than on the right side. It seems reasonable to assume that for some hydrodynamic reasons, the left carotid artery is more exposed to the stream from the aortic arch than is the right. There is some evidence of a similar bias in the laterality of cerebral embolies, as set forth in a previous paper by one of us (Acta Med. Scand. *154*: 1 1956).

Summary

Attention is called to the fact that the systolic murmur in aortic stenosis as projected into the carotid arteries is usually louder and more pronounced on the left side, although exceptions exist.

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From the Medical Department B (Head: John. Boe, M.D.), School of Medicine
University of Bergen, Norway

Clinical Course, Complications and Mortality in Typhoid Fever as Compared with Paratyphoid B

A Survey of 2,647 Cases

By

H. Gadeholt and Sigv. Trygve Madsen

Untreated typhoid fever is usually a much more severe disease than paratyphoid B, running a heavier course with more frequent complications and a higher mortality. In the literature, however, we have not been able to find comparative studies in this field, as at present such studies are made difficult by disturbing antibacterial treatment.

We have therefore found it of value to analyse the material at the University clinic, Bergen, Norway, in order to throw some light on the relative frequency and prognostic significance of complications in typhoid and paratyphoid B fevers in which antibiotic treatment had not been used.

Material

The patients were admitted to hospital from Bergen and surrounding districts during the years 1912 to 1961. The diagnosis was based on the clinical picture plus one or more of the following criteria:

1. Positive blood culture.
2. Significantly high or rising antibody titre.
3. Presence of typhoid, or paratyphoid B bacilli in stool or urine (carriers excluded).

The material includes 1 119 cases of typhoid fever: 545 males and 574 females. 137 carriers and convalescents hospitalized during the same period are excluded as well as 11 cases treated with antibiotics.

The number of paratyphoid B cases amounted to 1,528, including 684 males and 844 females. 65 carriers and 26 patients treated with chloramphenicol are excluded.

In about half of the cases, treatment included injections of different vaccines and non-specific proteins. There was no difference between the clinical course of the disease in treated and untreated cases.

Incidence

During the first years of the period, the notifications of typhoid and paratyphoid B in Western Norway were considerable and rose to 590 in 1918 (5). In the following years, 2 epidemics of paratyphoid B occurred in Bergen (1921 and 1925), but with these exceptions the number of cases reported showed a rapid decline, with paratyphoid as the more common of the two. These trends are clearly illustrated by the number of cases hospitalized in the five decades of our survey (table I).

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Table I Number of cases hospitalized in the Bergen area from 1912 to 1961

Years	No. of cases	
	Typhoid	Paratyphoid B
1912–1921	833	885
1922–1931	199	433
1932–1941	62	128
1942–1951	15	44
1952–1961	10	38
1912–1961	1 119	1,528

Table II Mean age in survivors and deceased

	Typhoid	Paratyphoid B
	Yrs	Yrs
Survivors		
♂	25 /12	21 10/
♀	25	23 /
Deceased		
♂	29 11/	35 10/
♀	31 /	34 4/

Table III Age distribution and mortality

Age groups (yrs)	Typhoid		Paratyphoid B	
	No. of pat.	Mortality (‰)	No. of pat.	Mortality (‰)
≤10	181	5.5	238	0.8
11–20	267	10.1	476	1 7
21–30	337	11 0	472	2.5
31–40	162	16.7	203	1.5
41–50	104	22.1	71	1 4
≥51	68	25.0	68	14 7
Total	1 119	12.1	1,528	2 3

Age distribution and mortality

In the typhoid group the youngest patient was 8 months, the oldest 74 years, in the para typhoid B group ages from 3 weeks to 83 years were noted. Mean ages in the two diseases are recorded in table II, showing a higher mean age in the deceased than in survivors. This difference is greater in paratyphoid B than in typhoid, amounting to 12 1/6 years and 5 1/12 years respectively both sexes included. This finding indicates that paratyphoid B infection is comparatively milder than typhoid in young people.

A similar trend is illustrated in table III In paratyphoid B a rapid rise in mortality is observed in ages over 50 years, whereas in typhoid the increase starts at the second decade.

The difference in mortality is significant, amounting to 12.1 in typhoid and 2.3 in paratyphoid B. From England Hutchinson (2) refers 12 % and 1 % respectively

Sex distribution and mortality

Of the 1 119 typhoid cases, 48.8 were males of the 1,528 paratyphoid B cases this percentage was 44 8 This corresponds well to the male population in Bergen, which is estimated as 45 6 % of the total population for the period concerned.

In typhoid fever the mortality among females amounted to 10.6 % (61 cases) among males to 13 6 % (74 cases) In para typhoid B the corresponding numbers were 2 5 (21 cases) and 2 0 % (14 cases) In typhoid there is a slightly higher mortality among the males than among the females. This difference was also noted by Stuart and Pullen (4) In paratyphoid B, the number of deaths is too small for reliable estimations in this respect.

Duration of fever

This investigation includes only survivors in whom exact observations concerning temperature were available, amounting to 984 cases of typhoid, 1,324 cases of paraty phoid B Termination of fever is defined as the first day with a maximum temperature below 37 5 C (rectal temperature) (tabl IV)

In the greater number of the typhoid patients, the fever lasted from 2 to 6 weeks (maximum 138 days) with an a erage of 30.2 days, in good conform ty w th th find ings of Stuart and Pullen (4) (31 5 day) In the paratyphoid B group, maximum duration of fever was 52 days, whereas the erage duration of fever was limited to 20 7 da s. This difference between th two diseases is significant. No d fference between the sexes was noted.

Table IV Duration of fever

Duration of fever (weeks)	Percentage of patients recorded	
	Typhoid (984 cases)	Paratyphoid B (1,324 cases)
< 1	0.3	2.1
1 – 2	5.5	16.6
2 – 3	16.2	34.4
3 – 4	26.6	35.2
4 – 5	22.9	9.6
5 – 6	13.1	1.8
6 – 7	5.5	0.1
7 – 8	4.4	0.2
8 – 9	2.4	
9 – 10	1.0	
> 10	1.9	

As for the duration of the fever in the different age groups, slight increase was observed with increasing age in typhoid fever whereas in paratyphoid B no difference was observed (table V)

Relapse

I reviewing the literature we could find no exact and generally accepted definition of relapse. Marmion (3) defined relapse as return of fever of 100° F or more, lasting 2 days or more, accompanied by any symptoms attributable to typhoid fever unless some other adequate cause could be found to account for the episode. W found this definition satisfactory with the exception that w have insisted upon an increase of fever to 38.0° C.

I typhoid fever relapses were noted in 16 (87 cases) of the males, and in 20 9 (120 cases) of the females, making an average of 18.5 %

In paratyphoid B relapses occurred in 3 8 of males (26 cases) and in 3 2 of females (27 cases) with an erage of 3 5

Relapses consequently are much more common in typhoid fever than in paratyphoid B (table VI) No sex difference was observed.

The age distribution of relapses is recorded in table VI There seems to be decline in the frequency with increasing age.

The duration of relapses in typhoid fever ranged from 2 to 37 days, average 11.5 days.

Table V Duration of fever in different age groups

Age groups (yrs)	Mean duration of fever (days)	
	Typhoid	Paratyphoid B
0 – 20	28.3	20.6
21 – 40	32.2	20.8
≥ 41	33.1	20.7
Mean total	30.2	20.7

Table VI Age distribution in patients with relapse

Age groups (yrs)	Typhoid		Paratyphoid B	
	♂	♀	♂	♀
	Percentage of relapse			
0 – 20	19.2	22.3	4.5	4.2
21 – 40	12.8	21.8	3.5	2.5
≥ 41	16.9	14.6	1.8	2.5
Total	16 0	20.9	3.8	3.2

Table VII Duration of relapse

Duration (days)	Typhoid (207 cases)	Paratyphoid B (53 cases)
	%	%
2	4.8	0
3 – 4	9.7	3.8
5 – 6	13.5	15.1
7 – 8	14.0	22.6
9 – 10	11.6	17.0
11 – 12	8 7	11.3
13 – 14	8.7	3.8
15 – 16	7.2	7.5
17 – 18	5.3	7.5
19 – 20	3.3	3.8
21 – 22	4 3	1.9
23 – 24	0.5	1.9
≥ 25	6.3	3 8

In paratyphoid B the range was from 3 to 37 days, average 11.3 days (table VII)

The afebrile period before return of fever ranged from 1 to 47 days in typhoid (average 6 days) from 1 to 41 days in paratyphoid B

Table VIII Duration of afebrile period before onset of relapse

Afebrile period (days)	Typhoid (*07 cases)	Paratyphoid B (53 cases)
	%	%
1— 2	30.9	39 6
3— 4	15.0	20.8
5— 6	16.4	7.5
7— 8	15 0	7.5
9—10	8.2	11.3
11—12	5.3	1.9
13—14	4.3	0
15—47	4.8	11.3

Table IX Time of onset of first gross intestinal hemorrhage

First bleeding observed (weeks)	Typhoid (139 cases)	Paratyphoid B (67 cases)
	%	%
0—1	0.7	0
1—2	20.9	50 7
2—3	41 7	34.3
3—4	20.9	9.0
4—5	5.8	1.5
>5	7.2	4.5
Unknown	2.9	0

Table X Nervous system complications

	Typhoid		Paratyphoid B	
	No of pat.	%	No. of pat	
Psychosis	11	1	2	0.1
Meningoencephalitis	1	0.1	1	0 1
Polyneuritis	4	0 4	3	0.2
Acute retention of urine	8	0.7	2	0.1

(average 6.6 days) The distribution of the length of the afebrile period is seen in table VIII

Regarding the duration of the relapse and the afebrile period there is consequently no difference between typhoid and paratyphoid B.

Two relapses occurred in 2 % (22 cases) in typhoid and in 0.3 % (5 cases) in paratyphoid B. Three relapses occurred in 3 cases of typhoid and in 1 case of paratyphoid B.

Gross intestinal hemorrhage

Gross intestinal hemorrhage occurs in untreated typhoid fever in frequencies ranging from 6 7 % (1) to 21 1 % (4)

In our typhoid group intestinal bleeding occurred in 12.4 % (139 cases) with a mortality of 25.2 % (35 cases) In the parathyphoid B group bleeding was recorded in 4 4 % (67 cases) with death in 17.9 % (12 cases)

The majority of cases were recorded in the first decades of the period investigated, and the lack of modern transfusion service obviously counts for the high death rate from intestinal hemorrhage The percentages referred to in typhoid and paratyphoid B are comparable and reflect the natural course of the two diseases. They indicate a much higher frequency in typhoid than in paratyphoid B with regard both to intestinal hemorrhage and to mortality from this complication.

Comparison between frequency of lethal bleeding and age gave conclusive differences between the age groups. In patients below the age of 10 years, gross intestinal hemorrhage was rarely observed, the frequency in typhoid being 1 7 in paratyphoid B 1 3 In no case was the hemorrhage fatal. At older ages no difference between the age groups was noted Mean age in lethal bleeding amounted to 29 years in typhoid, to 38 years in paratyphoid B

Hemorrhage most frequently occurred in the third week in typhoid, in the second week in paratyphoid B (table IX) The mortality from hemorrhage was the same in early and in lat hemorrhage.

Thrombophlebitis

Marmion (3) and Stuart and Pullen (4) found this complication in nearly 1 of their cases. In the present material thrombophlebitis occurred in 3.2 in the typhoid group, and in 2 4 in the paratyphoid B group. There was no sex difference but a slight increase of frequency with increasing ge

Table XI. Cause of death in typhoid and paratyphoid B fevers

Cause of death	Typhoid			Paratyphoid B		
	♂	♀	%	♂	♀	%
Intestinal hemorrhage	22	13	25.9	4	8	34.3
Perforation, peritonitis	12	7	14.1	2	1	9.0
Ruptured abscess, peritonitis				1	1	
Septicemia, toxemia	17	20	27.4	2	5	20.0
Pneumonia, bronchopneumonia	8	9	12.6			
Pulmonary empyema, abscess, gangrene	1	2	2.2			
Abscess of kidney	5	1	4.4			
Cholecystitis, empyema, perforation, gangrene		2	1.5			
Myocarditis	4	2	4.4	1	2	11.4
Pyonephrosis				1		
Thrombosis of superior sagittal vein					1	
Treatment with typhoid vaccine	1		0.74			
Cause of death precipitated by typhoid fever	4	5	6.66			
Cause of death precipitated by paratyphoid B fever				2	2	
Unknown				1		
	74	61		14	21	

reaching 7.2 % in typhoid males over the age of 41 years.

The mean age of the thrombophlebitic patients was higher than in the total material, reaching 31 years in males and 35 years in females (typhoid group), 25 years in males and females in the paratyphoid B group. The youngest patient with this complication was 13 years old male in the paratyphoid B group.

In the typhoid group the first sign of thrombophlebitis was noted on the 41st day in males (range 20—64 days) on the 30th day in females (range 11—70 days). In paratyphoid B the corresponding numbers were 29 day (range 18—49 days) and 28 days (range 13—49 days).

Genito-urinary system

Complications from the genito-urinary tract occur infrequently but they are not rarities. Stuart and Pullen (4) found pyelonephritis in 1.94 % and cystitis in 2.5 %.

In the present material, urinary tract infections were present in 2.6 (30 cases) in the typhoid group, and in 0.7 % (11 cases) in the paratyphoid B group. In the septic stage of the infection, symptomless bacilluria occurred in several cases. The exact number of these cases is however unknown as routine examination of the urine in symptomless cases were not carried out. One patient became urinary carrier.

In both groups the frequency of urinary tract infection was twice as high in females as in males.

In the typhoid group further 14 cases of nephritis, 4 cases of orchitis (2 of them children) and 1 case of hematuria were observed. Similar complications were not observed in the paratyphoid B group.

Nervous system

The frequency of these complications is seen in table X. Psychosis was the most frequent complication. Mental disturbances during the acute stage are excluded. Only complications later in the disease are noted, including paranoia, melancholia, hysteria, delirium, and non-specified psychoses. In two cases the psychosis became permanent, one ended in suicide.

Two of the polyneuritic cases were very severe with extensive paresis, but all recovered completely.

Transient deafness was a common complaint in both groups, but as routine examination was not carried out, exact numbers are not available.

Bones, joints and muscles

According to the literature these complications are uncommon. Stuart and Pullen (4) found 1 case of periostitis of a rib (0.28 %) Huckstep (2) 2 cases of osteomyelitis of tibia and femur (0.8 %)

In our typhoid group periostitis occurred in 2.1 % (25 cases) Half of these complications were noted in ribs and tibia. Other locations were clavicle metacarpal bones, iliac crest, femur fibula and metatarsal bones. Symptoms of periostitis started on the 41st day (range 31—58 days) in males, on the 37th day in females (range 22—60 days) In a 2 years old boy purulent typhoid arthritis of the cubital joint developed on the 38th day

Myositis was observed in 10 cases, staphylococcal periostitis in 1 and rheumatic fever in 1 No sex difference was observed

In paratyphoid B similar complications were still more infrequent. Periostitis was noted in 0.33 % (5 cases) located to ribs, tibia and fibula. One case of myositis was observed.

Pregnancy

In the typhoid group 9 patients were pregnant. Spontaneous abortion occurred in 4 and was provoked in 1 Pregnancy was unaffected in 3 normal labour occurred on the second day of the disease in 1

In the paratyphoid B group 9 patients were pregnant. There were no abortions. Normal labour occurred in all, in 5 of them during the 3 first weeks of the disease, in the rest labour occurred during convalescence or later

Discussion

Cause of death in typhoid fever (table XI)

Autopsy was performed in all cases with the exception of eight Four of these died of intestinal hemorrhage, and four with signs of severe toxemia.

The mean age of males and females is seen in table II The duration of the disease ante mortem ranged from 6 to 76 days with an average of 27 days There was no sex difference.

The higher mortality in males (13 6 %) than in females (10 6 %) may be due to the higher frequency of gross intestinal hemorrhage and perforation with peritonitis.

Bleeding is a serious prognostic sign. In Stuart and Pullen's (4) material 56 52 % of the patients with gross intestinal hemorrhage died in the present material the percentage was 43.2 Gross intestinal hemorrhage was the immediate cause of death in 25 9 % (Stuart and Pullen 15 21 %) The age distribution ranged from 14 to 55 years with an average of 29 years. There was no sex difference. The patients died between the 13th and the 41st day of the disease, the average for males being the 20th day for females the 27th day In five cases the hemorrhage occurred immediately after enemas given between the 14th and the 38th day of the disease, followed by death after hours or a couple of days

Perforation and peritonitis were cause of death in 14 1 % (Stuart and Pullen 10 87 %) There were 19 cases, 6 of them had operations and all of them died The age ranged from 2 to 64 years with an average of 29 1/2 years. No significant sex difference was observed Death occurred between the 11th and the 66th day averaging the 27th day in males and the 35th day in females. Simultaneous complications were bleeding (6 cases) bronchopneumonia (5 cases) gas gangrene tuberculosis and abscess of the mandible, one case each

Sepsis — toxemia was the diagnosis in toxemic cases in which no other adequate cause of death could be found The age ranged from 14 to 73 years and death occurred between the 6th and the 76th day averaging the 23th day There was no significant sex difference Contributory causes of death were recorded in 11 cases including cardiac pulmonary renal suprarenal (hemorrhage) and cerebral complications.

Pulmonary complications occurred in 38.5 % of the deceased, but was thought to be cause of death in 12.6 %. These were toxemic. The age of the patients ranged from 3 to 59 years with an average of 29 years. Death occurred between the 3rd and the 59th day with an average on the 21st day in males, on the 27th day in females. In three of these cases salmonella typhi was demonstrated in bronchial secretion, and in one of the three cases there were no signs of enteric affection the bacillus was, however cultivated from spleen and gallbladder. Another patient developed gangrene of the lung and splenic abscess.

Injection of typhoid vaccine was cause of death in a 45 years old male. He had been ill for 10 days, vaccine treatment was instituted, and he died 2 hours after the injection. At autopsy hematomata in the suprarenal glands were noted.

Degenerative changes of the kidneys were noted in 26.4 % (34 cases) nephritis in 2 cases, and pyelonephritis in 6. These changes were not believed to be the immediate cause of death.

Myocardial involvement was recorded in 12.6 % (16 cases) and considered to be the cause of death in 4.4 % (6 cases).

Other causes of death were embolic disease (one of them with abscess formation) (4 cases) noma gangrenosum of the face with gangrene of an arm and abscess of a leg + intestinal hemorrhage and pyelonephritis (1 case) hemorrhagic diathesis (retroperitoneal hematoma) (1 case) adenitis colli and phlegmone (1 case) and tuberculosis of the lung (1 case). One refused to take food (insane epileptic).

Cloudy swelling was a frequent finding in the internal organs, especially in cases recorded as toxemia and is probably of importance as attributable cause of death.

Cause of death in paratyphoid B fever (table VI)

Autopsy was performed in all but 5 cases. Of these intestinal hemorrhage was the cause of death in 3, toxemia probably in 2.

Duration of fever from time of onset of disease to death varied between 10 and 162 days.

Gross intestinal hemorrhage was the immediate cause of death in 34.3 % of the deceased (12 cases) with an age distribution of from 19 to 67 years, average 38 years.

Perforation with peritonitis occurred in 9 %, which is in conformity with the typhoid group (14.1 %). In 2 of these cases intestinal hemorrhage was present.

Pulmonary complications were present in 12 cases but was supposed to be cause of death only in one (see below).

Myocardial involvement was observed in 4 cases and was suspected to be the cause of death in all of them.

Cloudy swelling was a common finding, in 12 cases it was marked.

In 4 cases the acute paratyphoid B fever or its complications was *not* believed to be the immediate cause of death. An 83-year-old male did not recover his general health after the infection and died on the 162nd day of bronchopneumonia. A 73-year-old male with chronic nephritis developed a uremia in the acute stage of the fever and died subsequently in uremia. A 20-year-old female succumbed in paratyphoid B fever shortly after acute rheumatic fever with heart involvement. The attack of paratyphoid B was mild. In the convalescence she developed a fibrinous pericarditis and died of cardiac insufficiency on the 83rd day. An 18-year-old female with pulmonary and intestinal tuberculosis died on the 76th day after onset of the paratyphoid B fever.

Summary

A comparative study of untreated typhoid and paratyphoid B fevers is presented comprising 2 647 cases hospitalized during 50 years in Bergen, Norway

Age and sex distribution in the two disease groups roughly reflects the distribution of the population in the subject area.

The mortality from typhoid fever (averaging 12.1 %) increased with increasing age from the second decade, whereas in paratyphoid B fever an increase in mortality (average 2 3 %) only was observed in ages above 50 years.

The duration of fever varied in the two diseases, averaging 30.2 days in typhoid, 20 7 days in paratyphoid B

Relapses occurred in 18 5 % in the typhoid group in 3 5 % in paratyphoid B More than 1 relapse was noted in 2.1 % in the typhoid group in only 0 4 % in paratyphoid B Duration of the relapse was the same in the two groups (11.5 and 11.2 days, respectively)

Gross intestinal hemorrhage occurred in 12 4 % of the typhoid cases as compared with 4 4 % in paratyphoid B The mortality from this complication amounted to 25.2 % in the former to 17 9 % in the latter

Intestinal perforation and peritonitis occurred in 14 1 % and 9 0 % in the respective groups All of them died.

Main causes of death in typhoid were septicemia and toxemia, intestinal hemorrhage, peritonitis and pulmonary infection (complications) in the order mentioned In paratyphoid B death from intestinal hemorrhage was the major cause of death, with septicemia and toxemia peritonitis and myocarditis following in the order mentioned.

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A Study of Undiagnosed Myocardial Infarctions

By

F. Melichar, V. Jedlička and L. Havlík

In 1950—60 we observed an increasing number of cases of myocardial infarction (i.m.) in the clinical material of the 2nd Medical Department in Brno (13). This was also found in the autopsy material of the Department of Pathological Anatomy of the Medical Faculty in Brno (Head J. Švejda, M.D.) (15) where infarct of the myocardium was found at autopsy in 3,329 deceased patients out of a total number of 25 163 dissected bodies, i.e. in 13.2 per cent of all cases. Recent infarcts were found in 5.7 per cent of all dissected bodies. Since ever-increasing attention has been devoted in the past years to the diagnostics of i.m. (3—6, 10—18, 21) the authors have conducted a comparative study in order to determine the number of unrecognized infarctions for the years 1950—1960. Results are given in table 1.

Owing to the fact that only 59 per cent of recent i.m. and 28 per cent of old i.m. were diagnosed on clinical examination, the authors have decided to make an attempt to discover the chief reasons contributing to the fact why infarction of the myocardium was not diagnosed. First they studied all cases in which i.m. was not diagnosed clinically in the years 1959—1960.

Material and methods

From the autopsy records of the Department of Pathological Anatomy were selected all cases of recent i.m. or those in which a scar after healed i.m. was found, but all minor intramural and subendocardial i.m. were disregarded. All cases in which i.m. had not been diagnosed clinically or where i.m. had not been suspected, were considered to be unrecognized cases of i.m. The authors did not include in their study cases of old i.m. recorded in the patient's anamnesis or electrocardiogram but not given in the final diagnosis. Cases where both recent and old i.m. had been found at autopsy were classified in the group of recent i.m. In the autopsy records for 1959—1960 there were found 145 recent and 253 healed i.m. that had not been recognized clinically. In all these cases the authors evaluated the course of disease after studying the case histories that had been lent to them by the respective departments. The following points were evaluated: A. general data, B. antecedent history, C. history of the present condition, D. basic clinical diagnosis, E. number of myocardial infarction cases that could have been diagnosed clinically, F.

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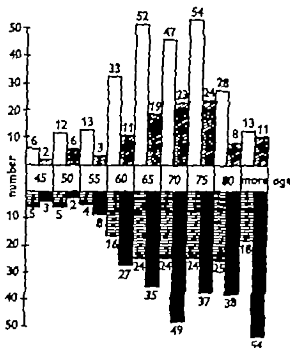


Fig 1 Distribution of recognized and unrecognized i.m according to age.
☐ Recognized recent i.m.
Recognized old i.m.
Unrecognized recent i.m.
■ Unrecognized old i.m.

pathologico-anatomical and clinical findings in patients who died and whose myocardial infarction had not been diagnosed

A. GENERAL DATA

1 Age Age groups of patients who died and whose i m. had not been diagnosed are given in fig 1 from which it follows that a total of 45 % of recent and of 51 of healed unrecognized i.m. was found in patients over 70 years. The mean age of all patients with undiagnosed i.m. is 70.8 years, i.e 69 6 years (fresh infarction) and 71 5 years (healed infarction) which is 5 years higher than in the group of recognized i.m. where the mean age was 65 8 years. Consequently age is one of the factors bearing on the accuracy of diagnosis in myocardial infarction (4 15)

2 Sex The ratio of men to women with unrecognized i.m. is 1.53 1 while in the group of recognized i.m. it is 2.37 1 which indicates that i.m. in women is not recognized as often as in men. This is partly due to the fact that i.m. occurs in women at a higher age than in men (16, 17)

3 Localization of myocardial infarction. In most cases, i.e. in 54 % infarction was found in the posterior wall (9 13) Infarcts were localized in the anterior wall in 13 % in the anteroposterior wall in 10 % in the anteroseptal wall in 8 in the postero-anteroseptal wall in 6 % in the posteroseptal wall in 3 % and in other areas in 6 of all investigated cases. At the same time the percentage of unrecognized i.m. grouped according to site of infarction is also the highest in infarcts of the posterior wall, constituting 60 % of all infarctions of the posterior wall found in 1959—1960 Next come infarctions of the anterior wall with 49 % and those of the anteroposterior wall 42 Since infarction of the posterior wall most frequently escapes clinical diagnosis and usually predominates among healed infarctions, it may be assumed that its course is frequently atypical.

B ANTECEDENT HISTORY

The antecedent history revealed angina pectoris, atherosclerotic non-cardiac complications, hypertension, diabetes and previous diseases of the heart. In 93 patients there was no past history of illness or the anamnesis was not sufficiently detailed Of the remaining 305 cases an anamnesis indicating angina pectoris appeared only in 5.9 % of cases. Hypertension (blood-pressure over 150/95) was reported in the past history of those who died and whose i.m. had not been diagnosed in 35 5 which is considerably higher than in patients with recognized i.m., where hypertension was reported only in 10 9 of cases. Similarly diabetes occurred more frequently in those with unrecognized i.m. (10.6 %) than in patients with diagnosed i.m., where it amounted only to 2 4 Previous cerebro-vascular accidents were reported in 12 5 % In 41 % of the cases investigated there was a past history of heart disease for which the patient had been treated for a longer period.

Table II shows that no heart disease atherosclerotic complications, hypertension and diabetes were reported in the past history of 31 6 % of those who died and whose i.m. had not been recognized.

C. HISTORY OF THE PRESENT CONDITION

In the patient's description of the symptoms of his present condition the authors found that only in 10 of recent and 1 5 of old i.m.

Table I. Percentage of unrecognized i. m. found in the autopsy records for the years 1950—1960

Year	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960
Recent i. m.	59	64	49	54	46	36	28	44	40	31	38
Old i. m.	83	87	76	86	92	58	77	74	70	74	67

had the patients complained of anginal pain as one of the outstanding symptoms of the present condition. In further 8 of patients with unrecognized recent infarction retrosternal pain was not usually marked apart from predominating symptoms of some other illness. In 35 % of patients congestive heart failure was prevalent. The percentage of cerebrovascular accidents is also high. On the basis of these symptoms 15 % of patients had been admitted in whom recent infarction of the myocardium was revealed upon autopsy while old infarcts were found in 22 %.

In those who died with recent unrecognized i.m., the authors also noted their general condition upon admission to hospital and the time which elapsed between their admission and death. Seventeen patients died within 24 hours, 16 patients within 48 hours and 9 within three days after being admitted to hospital. Twenty-two other patients survived this period but their condition upon admission was very serious. Thirteen other patients died suddenly during treatment for some other illness. These patients could not be examined completely. From the afore-mentioned it follows that even the onset of recent i.m. in the majority of patients with unrecognized cardiac infarction was not accompanied by typical anginal pain. This was another significant factor that contributed to the infarction being unrecognized (7 11). The serious condition of the patients and the predominance of signs and symptoms indicating the presence of some other illness was further factor resulting in the cardiac infarction being clinically unrecognized.

D BASIC CLINICAL DIAGNOSIS

The most frequently given fundamental diagnosis was congestive heart failure namely in 43.4 % (in 31 % of recent and 42 % of old i.m.) Then follow acute cerebro-vascular accidents (in 19 of cardiac infarctions) tumours (12 %) and pulmonary embolism

Table II. Antecedent history of patients who died with unrecognized i. m.

	Recent i. m.	Healed i. m.	Total No.
	143	233	398
Angina pectoris	4.3	7.1	5.9 %
Hypertension	34.0	36.2 %	35.4 %
Heart disease	45.7	38.8 %	41.0 %
Arteriosclerotic cerebral complications	12.2	13.2 %	12.5 %
Diabetes	14.6 %	8.4 %	10.6 %
Negative data	27.2 %	33.4 %	31.6 %
Anamnesis missing	29	64 x	93

Table III. History of present condition

	145 i. m. recent	233 i. m. healed
Anamnesis missing	7	6
Anginal pain	10	1.5 %
Anginal pain and some other disease	8 %	2.7
Congestive heart failure	35	33
Non-cardiac disease	34 %	41 %
Acute cerebro-vascular lesion	15 %	22 %

(5.2 %) Surgical illnesses (3.5) renal diseases (3.7 %) diseases of the lungs (3.5 %) various infirmities of old age (1.7 %) and TB of the lungs (1.5) are also diagnosed. The given basic diagnosis agreed in most cases with the finding of the pathologist. Pulmonary embolism (in 6 cases) and sudden onset of cerebro-vascular accidents (in 8 cases) were incorrectly given by the doctor as the cause of death, although, in fact, in all these cases recent infarction of the myocardium was involved.

Other incorrect basic diagnoses included 6 cases of decompensated heart disease not

Table IV

	Recent i. m.		Healed i. m.	
	No.	%	No.	%
Unrecognized i. m. and fresh cerebro-vascular accidents	26	17	55	21.5
Unrecognized i. m. and massive pulmonary embolism	9	6	16	6.5
Unrecognized i. m. and serious noncardiac disease	33	22	93	37.5
Unrecognized i. m. and cardiac disease.	77	55	87	34.5

proved by autopsy and one case of ruptured aneurysm of the aorta. Acute heart failure without marked previous decompensation was found only in 1 5 % of the cases investigated. Fresh decompensation of the heart as the only sign of infarction of the myocardium (14) was found in the group of patients with recent i.m. in one case only At the same tim., unrecognized i.m. was very often accompanied by a deterioration of the cardiac decompensation for which the patient had been previously treated. In these patients, however no coronary pain had been reported and the typical ECG changes were also missing, so that the decompensation was attributed to the basic disease, in most cases to chronic ischaemic heart disease, hypertension, cor pulmonale chron. or cardiac valvular defects.

E. NUMBER OF MYOCARDIAL INFARCTION CASES THAT COULD HAVE BEEN DIAGNOSED CLINICALLY

On the basis of the past history the condition of the patient on admission to hospital, ECG information, changes in the sedimentation rate and the leucocyte count of the patients with unrecognized i.m., the authors inferred whether the i.m. could have been recognized with certainty or whether at least, justifiable suspicion could have been expressed in the diagnosis. We have found that on the grounds of the past history or of typical ECG changes and of laboratory investigations, i.m. could have been recognized in 7 patients with recent and in 2 patients with old i.m.

ECGs suspected of i.m. changes were found in 28 cases (13 fresh, 15 old i.m.) including 11 patients with preceding retrosternal pain. In most of these cases only routine records through 3—4 leads had been taken. Left bundle branch block with suspected coronary anamnesis existed in four cases only with recent i.m. Consequently out of 145 recent i.m. and 253 old i.m. a correct diagnosis could have been arrived at in 24 and 17 cases respectively or at least, justifiable suspicion could have been expressed by the diagnostician. In the remaining cases, i e. those who died and whose i.m. had not been recognized, infarction was not indicated either by the past history or by the clinical picture.

F. PATHOLOGICO-ANATOMICAL AND CLINICAL FINDINGS IN PATIENTS WHO DIED AND WHOSE MYOCARDIAL INFARCTION HAD NOT BEEN DIAGNOSED

On the basis of autopsy and clinical findings the cases were divided into four groups, as shown in table IV

1 Recent unrecognized i.m. and fresh cerebro-vascular lesions

Included in this group are those cases in which recent i.m. and recent encephalomalacia or haemorrhage were concurrently found on autopsy so that in most cases it was not possible to decide which of the above causes was the primary or direct cause of death. At the same time, the cause of the cerebro-vascular lesion was not possible to determine. Such a coincidence of these 2 events occurred in 17 of unrecognized i m. Hypertension was present 15 times, i.e. in 57 In two cases cerebral embolism was involved due to mitral disease, while in the remaining 8 patients the blood pressure was normal after the cerebro-vascular and heart disorder but it was not possible to establish their earlier blood pressure Consequently t may be assumed that hypertension occurred in even more of these cases. Included in this group are also 55 patients (21 %) in whom healed i.m and recent encephalomalacia was found on autopsy Hypertension was established in 67 of all cases.

Hypertension occurred in this group much more frequently than in the group of unrecognized i.m. (35 4 %) as well as recognized cases of i.m. (10.9)

2. Recent myocardial infarction and embolism of the pulmonary artery

There were 9 patients (6 %) at whose autopsy recent i.m. was established apart from massive pulmonary embolism, which was also the cause of death in 16 patients (6) with healed i.m.

Moreover in further 5 patients not included in this group pulmonary embolism was established in advanced malignant disease while in 5 patients pulmonary embolism was found concurrently with old healed i.m. and fresh encephalomalacia.

3. Recent infarction of the myocardium and serious non-cardiac disease

Included in this group are 33 patients suffering from some disease not connected directly with the cardio-vascular system, but which itself would have shortly resulted in the patients death as well as recent i.m. Fourteen of these patients had some malignant condition, 6 patients suffered from some serious surgical affection and sepsis, 6 patients had diabetic nephrosclerosis, 2 of the patients suffered from uraemia of renal origin and advanced tuberculosis of the lungs, one from diabetic coma, bronchopneumonia with abscesses and purulent meningitis. The condition of all these patients was very poor and myocardial infarction was the terminal event without typical ECG changes and anginal pain, which was marked in only two patients of this group.

The authors further included in this group 95 (37.5 %) patients with healed i.m. In 45 of these patients the fundamental disease was some malignant process in an advanced stage with metastases. Eleven patients suffered from kidney disease in its final stage with azotaemia. The same number of patients had septic process of different origin. In 9 cases there was serious primary disease of the lungs and the pleura, while 10 patients suffered from acute abdominal disease and postoperative affection. Other less frequent causes of death of patients with healed i.m. were advanced tuberculosis of the lungs (2 patients) severe haemorrhage and injury (2 patients) hepatic cirrhosis (1 case) status epilepticus (1 case) and hyperthyroidism (1 case).

Altogether 22 % of these patients with unrecognized recent i.m. and 37.5 % with undiagnosed old i.m. suffered from some serious non-cardiac disease with the result that in most cases the clinical practitioner did not even suspect infarction.

4. Group of patients with unrecognized myocardial infarction and basic cardiac disease

In this group are included 77 patients with recent undiagnosed i.m. in which the main and direct cause of death was cardiac disease. Among these was a number of patients who had been for a long time treated for disease of the cardio-vascular system of origin other than coronary disease. In 7 cases this was some valvular defect, in 7 patients cor pulmonale chron. decompensatum, in one case purulent pericarditis. Further 5 patients were in a very poor condition after previously having suffered from cerebral affections. The authors further included in this group 87 patients with healed i.m. In 10 of these patients the base disease was cor pulmonale chronicum decompensatum, 9 suffered from a decompensated valvular defect, while in one case the cause of death was rupture of the aorta. Consequently in the group of patients dying of heart disease there was number of patients in whom healed i.m. was only an incidental finding (1).

Unrecognized myocardial infarction and ECG examination

ECG records were not taken in 72 deceased patients with recent i.m. and 131 patients with unrecognized healed infarction of the myocardium during their final period of hospitalization. In the group of recent i.m. there were 41 patients, while in the group of healed i.m. 82 patients whose ECG records were markedly pathological (diffuse muscle disorder left or right ventricular strain, auriculo-ventricular heart blocks, arrhythmia) without clear signs of i.m. Marked evidence of myocardial infarction was found on the ECGs of 4 recent and 2 old i.m. I further 13 recent and 15 old i.m. the ECGs were found to show suspected changes of infarction. Left bundle-branch block was present in 8 recent and 10 old i.m. In the group of recent infarctions normal ECGs were found in 2 cases, while in the group of old m. they were found in 13 cases.

From the above-mentioned it follows that another factor contributing to myocardial infarction remaining unrecognized is the fact that ECG examination had not been carried

Table V The wards where the patients were hospitalized

	Medical ward		Surgical and gynecological		Psychiatric		Oncological		Other wards	
	No.	%	No.	%	No.	%	No.	%	No.	%
Recent i.m.	96	66.4	23	15.8	14	9.8	4	2.6	8	5.4
Old i. m.	157	62.0	48	18.9	35	13.0	12	4.9	3	1.2

out in 51 % of all patients dying with unrecognized i.m. In the group of patients where ECG investigation had been carried out, the reason for non recognition of infarction was, apart from left bundle branch block, the fact that the patients ECGs were pathologically altered without showing typical infarction changes. Another factor is that very few ECG leads were taken in these cases (22)

In table V the wards where the patients *with unrecognized i.m* were hospitalized are given.

Discussion

The object of the authors study was to establish the most frequent reasons why in the years 1959—1960 the clinicians were not able to recognize 145 recent and 253 healed i.m They came to the conclusion that on the basis of a well balanced judgment of all the facts relating to the cases and a proper evaluation of all relevant data of the clinical picture only 10 per cent of myocardial infarctions could have been recognized and diagnosed or *at least suspected.*

One of the most frequent reasons why i.m. could not be recognized was its atypical painless course. Angina pectoris occurred in the past history of the patients with unrecognized i.m. only in 4 5 per cent of all cases investigated by the authors. Out of 145 patients who died with recent i.m only 18 per cent experienced anginal pain of varying intensity at the onset of infarction

This high number of painless i.m must have been caused by the relatively advanced age of the patients who died with unrecognized i.m. In the autopsy sheets studied by the authors nearly one half of the patients were over 70 years old the mean age of all patients being 70 8 years, i.e. 5 8 years higher than the mean age of all deceased patients aged more than 20 years, on whom post mortem examination was performed in the years 1959—1960 that is approximately 65 years. The fact that the number of unrecognized healed i.m predominates in patients over 80 years indicates that myocardial infarction is often only an incidental finding without any bearing on the prognosis of the patients involved (5)

With the advanced age of patients dying with unrecognized i m is connected their serious condition on admission to hospital or their sudden death, which prevents the physician from examining the patients properly In the group of recent i.m. there were 53 per cent of such cases.

The serious condition of the patients and particularly their sudden death is caused to a great extent, by the frequent occurrence of myocardial infarction with encephalomalacia and pulmonary embolism (2, 3 8, 12 20) In the materials studied by the authors of this paper i.m. and encephalomalacia occurred together in 17 per cent of recent and 21 per cent of old unrecognized i m The combination of these two affections together points to common aetiological

factors, such as atherosclerosis and hypertension, which was found in 50 per cent of the cases studied. A combination of pulmonary embolism and i.m. occurred in 11 per cent of cases. Signs and symptoms of these affections obscure the eventual manifestations of i.m., as shown by the fact that in the group of clinically diagnosed i.m. there was a combination of cerebro-vascular accident and i.m. only in 2.4 per cent of cases.

Hypertension too, occurs in the group of unrecognized i.m. more frequently (35.4 %) than among the clinically diagnosed cases (10.9 %) Consequently hypertension, also, contributes greatly to the fact that i.m. is not recognized probably owing to the fact that it leads to a more rapid development of atherosclerosis, heart failure and cerebro-vascular accidents.

Similarly diabetes occurs in the group of unrecognized i.m. much more frequently (10 6) than in the group of recognized i.m. (2.4 %) In these cases, however it was usually the final stage of diabetes with nephrosclerosis and hypertension.

In medical wards the most frequent disease for which patients with unrecognized i.m. were treated is cardiac decompensation (46 %) usually caused by chronic ischaemia of the heart (19)

Examination by electrocardiography which is undoubtedly the most important method yielding information necessary for the diagnosis of i.m., was not carried out during the last hospitalization in 51 per cent of patients. This was because of the sudden death, the poor condition of the patients, the predominant symptoms and signs of some other serious disease, and further the fact that nearly one half of the patients had been admitted to non medical wards. In the majority of the other patients only routine ECG leads were taken showing a pathological curve without marked changes of infarction

Another factor contributing to unrecognized i.m. is the fact that in non medical wards, or in the case of patients where the manifestations of another disease are clearly predominant the doctors concentrate their attention only on the anamnesis of the basic disease and overlook the symptoms and signs of coronary disease. This should never happen since in the case of elderly people, from whatever disease they may suffer the physician should always think of coronary disease.

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The reasons why myocardial infarction was not recognized may be summarized as follows

1 Out of 598 unrecognized cases of i.m. in the years 1959—1960 only 10 per cent could have been recognized
2. The chief reason why i.m was not recognized had been the painless onset and course of i.m. in 87 6 per cent of all recent unrecognized i.m.
3 Unrecognized i.m. occurred mostly in old people of a mean age of 70 8 years, of whom 50 per cent were older than 70
4 53 per cent of patients dying of recent myocardial infarction were admitted to hospital in a very poor condition or died suddenly without having been properly examined
5 i.m. could not be recognized because of the coincident occurrence of encephalomalacia in 17 per cent of recent and 21 per cent of old i.m. further by the incidence of pulmonary embolism in 11 per cent of cases
6. i.m. is more frequently not recognized in patients suffering from hypertension and diabetes

7 22 per cent of recent and 37 5 per cent of healed unrecognized i.m. were found in patients suffering from some serious non-cardiac disease,

8. Unrecognized i.m., both recent and healed is frequently only a component part of a chronic ischaemic disease of the heart manifesting itself by congestive heart failure for which the patients were in most cases admitted to hospital

9 ECG examination was not carried out in 51 per cent of patients who died of unrecognized i.m. while in the other patients an inadequate number of ECG leads were taken

10 In the anamnesis of elderly patients often suffering from some other disease little attention is paid to the less marked symptoms of coronary disease if the physician did not overlook them he could direct his attention to this serious disease

Summary

In the autopsy records of the Department of Pathological Anatomy of the Brno Medical School Hospital (for the years 1959—1960) the authors found 145 cases of recent and 253 healed infarctions of the myocardium that had not been clinically diagnosed They evaluated the course of the disease and arrived at the conclusion that only 10 per cent of i.m could have been clinically recognized The main reasons why myocardial infarction is not recognized are as follows atypical painless course, advanced age of patients their poor condition on admission to hospital occurrence of some fresh cerebro-vascular accident or pulmonary embolism. Myocardial infarction is frequently not recognized in patients suffering from hypertension and diabetes. It often escapes clinical diagnosis in patients suffering from some serious non-cardiac disease and in those with some degree of congestive heart failure. Inadequate ECG examination is also a factor contributing to the failure to recognise infarction of the myocardium.

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The Effect of Carbohydrate Restriction on Glucose Tolerance, Serum Insulin-like Activity and Growth Hormone-dependent Sulphation Factor in the Serum of Diabetics

By

S. Ekkjær Jensen, K. Lundbæk and J. Lyngsøe

Previous studies of the insulin-like activity of the serum in diabetic subjects have shown that two clinical types of diabetics have different levels of serum insulin-like activity (SILA). In non-obese non-acidotic diabetics, usually juvenile patients whose diabetes requires insulin, studies using the rat diaphragm method have shown that SILA was either low or absent in undiluted serum (13, 15), while SILA values corresponding to the level found in normal subjects were obtained by means of the rat epididymal fat method (11). Obese diabetics, usually older patients suffering from mild diabetes with no tendency to ketosis, have high SILA values with both the methods mentioned (11, 15). Recently immunological studies of the growth hormone content of serum have shown that the two diabetic types differ with respect to level of growth hormone, as untreated obese diabetics have apparently a higher blood content of growth hormone than untreated non-obese patients (4).

The studies cited above would seem to suggest that there is an essential difference between the disease called diabetes mellitus in obese and non-obese diabetics. In order to elucidate this question, the present investigation has examined the effect of carbohydrate restriction on intravenous glucose tolerance, SILA (using the rat epididymal fat method) and growth hormone-dependent sulphation factor (SF) in a number of recently-diagnosed obese and non-obese diabetics.

Material

The patients studied represent a random group of untreated, recently-diagnosed diabetics admitted to the medical department. On the day of admission the patients were instructed to follow perfectly normal, unrestricted diet. Blood sugar was determined daily at 7 a.m., 1 p.m. and 5 p.m., to establish the 24-hour blood sugar level. A few days after admission to hospital, blood samples were taken for SILA and SF assay and an intravenous glucose-tolerance test performed. The

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Table I Intravenous glucose tolerance (k) serum insulin like activity (SILA) and sulphation factor (SF) before and after carbohydrate restriction (non-obese diabetics)

Pt. no.	Sex	Age	Weight (%)	k value		Fasting serum glucose mg %		SILA, undiluted serum μU/ml		SILA, diluted serum μU/ml		SF	
				Before	After	Before	After	Before	After	Before	After	Before	After
2	♂	64	< 100	.37	.24	243	228	90	185	285	200	.85	.57
8	♂	30	< 100	.30	.23	216	198	265	95	435	90	.98	1.32
9	♀	71	111	.22	.32							.99	1.28
13	♂	70	108	.35	.30	199	131	185	205	285	475	.98	
15	♂	53	< 100	48	.39	263	*66	82	98	150	240	.90	
16	♀	60	< 100	40	.37								
17	♀	47	< 100	33	45	271	254	50	90	115	450		
18	♂	28	< 100	.34	.21	246	198	21	78	75	105	73	.90
20	♀	57	109	44	43	266	217	64	143	85	285	1.22	1 44
22	♂	67	111	.37	41	296	179	40	70	335	165		1.03
23	♀	31	< 100	.62	.94	185	112	108	118	80	425		
24	♂	17	< 100	40	.56	220	81	165	0	110	0		
26	♀	49	< 100	.37	.36	222	199	60	44	75	80		.61
27	♀	71	102	.34	42							1 00	1 11
29	♂	61	114	43	.57	156	130	13	245	0	280	1 08	79
32	♂	23	105									1.20	1 16
34	♂	20	< 100			241	170	0	155	0	215	.96	1.23
			Mean	0.38 ± 0.09	0.41 ± 0.18	232.6	181.8	88 ± 76	117 ± 68	156 ± 135	232 ± 149	.99 ± 14	1 04 ± .29
3	♂	69	< 100	.50		278		95		90		1.08	
5	♀	39	< 100	.28		280		170		315		.85	
7	♀	16	< 100	48		257		56		165		76	
8	♂	30	< 100	.30		216		265		435			
11	♂	23	< 100	.37		208		175		220		.96	
			Mean	0.38 ± 0.09		236.8		106 ± 81		181 ± 137		.97 ± .14	

patients were then put on a diet with a fixed and reduced carbohydrate content, achieved by excluding sugar and sweets and fixing the content of bread and potatoes. Most patients received a diet containing 100 g bread and 100 g potatoes per day. After dietary treatment for 1—2 weeks, a fall in the 24-hour blood sugar values was found in the majority of the patients. After these values had stabilized at a level which was clearly lower than that prior to the diet, blood samples were again taken for SILA and SF determination, and a further intravenous glucose tolerance test performed. The determinations were not repeated in those patients who showed no fall in the 24-hour blood sugar level following the diet.

The patients were divided into two groups, obese and non-obese diabetics. Obesity is defined as a weight which is ≥ 115 % of an ideal weight extracted from the "Hafnia" tables of mean weights for a normal Danish population aged 30—34 years (10).

The mean fall in 24-hour blood sugar values after the diet was of the same order of magnitude in both diabetic groups examined, 75 and 90 mg % for obese and non-obese diabetics, respectively. A weight loss during the period of observation was recorded in both groups, a mean value of 1.2 kg in the obese diabetics and 0.6 kg in the non-obese diabetics. A few of the non-obese diabetics suffered from ketonuria, but none of them had acidosis.

Table II. Intravenous glucose tolerance (k), serum insulin-like activity (SILA) and sulphation factor (SF) before and after carbohydrate restriction (obese diabetics)

Pt. no.	Sex	Age	Weight (%)	k-value		Fasting serum glucose mg %		SILA, undiluted serum μU/ml		SILA, diluted serum μU/ml		SF	
				Before	After	Before	After	Before	After	Before	After	Before	After
1	♀	64	117	14	.36								
4	♀	66	158	.27	.25	218	181	320	250	1,850	0	.81	43
6	♀	66	124	43	.52	220	112	450	165	550	195	.80	.84
12	♀	63	130	.58	46	198	128	100	44	565	185	1.06	
14	♀	58	162	.17	47	193	164	88	100	120	115	1.05	.88
19	o	57	130	.36	48	209	136	560	90	465	140	1.32	1.20
21	♂	60	143			208	176	145	54	90	85	1.28	
25	o	58	113	.39	.32	183	150	160	41	700	0	1.26	.92
28	♂	47	126			262	133	233	500	550	280	1.44	1.00
30	♀	4	159			216	120	175	122	725	260		
31	♀	63	117	.50	.30	193	135	100	130	500	65		
33	♂	51	117			247	168	132	11	210	0	0.90	1.13
35	o	54	118	75	.61								
36	♀	54	185	.36	47								
37	♀	71	125	.51	43								
38	♂	64	154	.32	40								
			Mean	0.40 ± 0.17	0.44 ± 0.09	213.4	147.5	224 ± 156	137 ± 138	584 ± 503	120 ± 106	1.11 ± 0.23	0.91 ± 0.23
								t = 1.370 p > 0.1		t = 2.897 p < 0.02		t = 2.182 0.1 > p > 0.05	
10	♀	63	148	.36		236		110		215		.91	
			Mean	0.40 ± 0.17		215.2		215 ± 152		553 ± 497		1.10 ± 0.22	

Methods

All diabetic subjects were examined between 7 a.m. and 9 a.m., after a fast for 8—12 hours and bed rest. The technique employed in the intravenous glucose-tolerance tests and in the calculation of the k-value was as indicated by Lundbæk (8).

Serum for SILA and SF assay was prepared from venous blood as on previous studies (10). SILA was determined by the rat epididymal fat method using fresh serum, both diluted and undiluted (9). The mean index of precision for the SILA assays in the present study is 0.25 ± 0.10.

The SF assays were made on serum samples stored at −20° C by Almqvist modification (1) of Daughaday et al.'s method (3). Costal cartilage was used from rats hypophysectomized transaurally by Tanaka method (16).

The assays were carried out by measuring in a symmetrical four-point system with two serum volumes of the laboratory reference serum (30-year-old man) and two volumes of the unknown serum, so that each biological measurement was self-contained and independent. All results were statistically valid, statistical control being kept of the significance of the regression and of the variation in slope (deviation from parallelism) as well as of the differences between the mean effect of the standard and unknown serum according to the formula provided by Borth et al. (2). The mean index of precision in this study was λ = 0.22 (± 0.07).

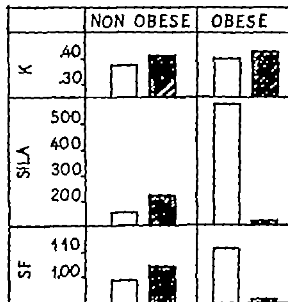


Fig 1 Average values of k, SILA and SF in the two groups of patients before (□) and after (▣) diet (for statistical significances, see text)

Results

In a previous study of SILA in diabetics, a higher SILA level could be demonstrated in obese patients than in non obese patients (11) In the present study therefore, the material of untreated diabetics has been grouped according to the same criteria as in this previous study

Unrestricted diet

Tables I II and fig 1 show that there is no difference in the intravenous glucose tolerance in the two groups of diabetics the mean k value in the non-obese diabetics being 0 38 and in the obese diabetics, 0 40.

On the other hand the fasting SILA values show a clear difference in the two groups of diabetics. The mean SILA value in undiluted serum is 106 μU/ml in the group of non-obese diabetics against 215 μU/ml in the group of obese diabetics ($p < 0.02$) The corresponding values for diluted serum are 181 μU/ml and 553 μU/ml ($p < 0.01$) The statistical analysis of these mean values thus shows that this difference between obese and non-obese SILA values is significant. The same is true of the difference between the values obtained in diluted and non-diluted serum, from diabetics as well as from non-diabetics ("Dilution effect" (10))

If the SF values in the two groups are compared the mean value is seen to be 0.97 in the group of non-obese diabetics, against 1 10 in the group of obese diabetics. This difference is however not statistically significant ($t = 1.754$ $0.05 < p < 0.1$)

Carbohydrate restriction

After a fall in the 24-hour blood sugar level could be recorded in the patients on diet, the intravenous glucose tolerance tests, SILA and SF determinations were repeated.

In the group of *non-obese diabetics* no definite change in k, SILA or SF values can be demonstrated after diet. The mean increase in k values is 8 % but this is not statistically significant ($p > 0.1$) The SILA values rise on the average 33 % and 49 % in undiluted and diluted serum, respectively but the rise is not statistically significant ($p > 0.1$) These SILA values show a dilution effect both before and after diet After diet the SF values rise on the average 5 % in non-obese diabetics, but neither is this increase significant ($p > 0.1$)

In the *obese diabetics* no change is observed in the intravenous glucose tolerance after diet (non-significant rise of 10) In the same group, on the other hand, there is a fall in the SILA values in both diluted and undiluted serum The mean fall in SILA in undiluted serum is 59 and in diluted serum 80 % of the value

prior to diet, but the fall is statistically significant only in the case of the diluted serum ($p < 0.02$). The SILA determinations following the diet show no difference of levels in the undiluted and diluted serum. The dilution effect which was present in the untreated obese diabetics has thus disappeared during carbohydrate restriction.

After dieting obese diabetics show a fall in the mean SF value equal to 18 % of the pre-treatment value and hardly significant statistically ($0.1 > p > 0.05$).

Discussion

In the present investigation, an attempt has been made to throw light on the metabolic and hormonal status in diabetes mellitus. The material consisted of obese and non-obese patients with diabetes of recent origin. The determinations of the k-value, SILA, and SF were performed on the patients before any form of treatment had been started, and they were repeated after a fall in the blood sugar level had been obtained by means of restricted carbohydrate ingestion.

An examination of the *untreated condition* (unrestricted diet) showed the state of affairs previously described, namely that SILA values in obese diabetics are higher than in non-obese diabetics. A tendency to higher SF level existed in the obese. The k values of intravenous glucose tolerance on the other hand, were the same in both these groups of patients.

Limitation of the carbohydrate supply resulted in a fall in the fasting blood sugar level, of the same order of magnitude in obese and non-obese patients. The metabolic anomaly as expressed by intravenous glucose tolerance test, remained however unchanged t the new lower blood sugar level and this was the case both for obese and non-obese patients.

On the other hand, the effect of reduced carbohydrate ingestion on SILA and SF differed in obese and non-obese subjects. In the group of obese patients, SILA showed a fall which was considerable and statistically significant in diluted serum, and SF showed a tendency to fall. These two hormonal parameters showed no significant change in the group of non obese patients.

The above findings stress the difference between diabetes mellitus of the obese and of the non-obese, and suggest a difference in pathogenesis. Although the findings elucidate the hormonal status in obese and non-obese diabetics, they do not permit any precise formulation of endocrine abnormalities in these two types of diabetes. Our knowledge of production, active serum concentration and consumption of insulin and growth hormone is too limited for such a task.

SILA determined by the rat epididymal fat method probably represents only part of the insulin content of serum. Previous studies have in fact shown that a considerable amount of insulin is present in serum in a form which cannot be recorded directly by the rat epididymal fat method (6, 12). It is uncertain whether SILA determined by this method reflects the amount of insulin available to the organism.

SF is not growth hormone but a factor of unknown nature, the concentration of which in the blood appears to follow the production of growth hormone closely (1). The growth hormone is known to have a diabetogenic effect in man (5, 7).

The high SILA of *obese diabetics* may be an expression of a high production or a reduced consumption of insulin, but it may also reflect an abnormal relation-

ship between the various states of insulin. The tendency to high SF is in accordance with the high growth hormone concentration which is demonstrated by an immunological method in obese untreated diabetics (4)

It is possible that in obese diabetics the diabetic state is due to an overproduction of growth hormone, causing elevated blood sugar level and that (by compensation?) this brings about a rise in the insulin production or a change in the state of the insulin in the serum. Under normal conditions it is known that SILA rises when the blood sugar level is raised (10 14) and Zahnd et al (17) have shown that SILA is increased four hours after injection of growth hormone, while simultaneously the blood sugar level shows a tendency to rise.

In obese subjects, a fall in blood sugar level produced dietetically causes a pronounced fall in SILA and the usual difference between SILA in undiluted and diluted serum disappears. Simultaneously a tendency is seen to a fall in SF

It seems reasonable to consider these changes as indicating a regulatory change in the hormone production or in the state of the hormones (insulin, perhaps even growth hormone) in the blood in response to a fall in the blood sugar level but at the moment it is not possible to go into further details of such a regulation

Non-obese diabetics have normal SILA and on the average a lower SF than obese diabetics. It is probable that in these patients there is no increased growth hormone production. The diabetic state may be due to a lack of insulin in the tissues, either as a result of reduced insulin production or because of an abnormality in the state of the serum insulin.

The absence of any change in SILA and SF following the fall in blood sugar level brought about by the diet may signify the disappearance of the above-mentioned regulatory mechanism of insulin production.

It is interesting that the metabolic abnormality as expressed by the glucose tolerance test does not alter when the blood sugar level falls. In obese patients, this may be due to a simultaneous reduction in the active insulin and growth hormone of the blood while in non-obese patients it may be that the activity of both those hormones continues unchanged

The present studies deal only with the state of affairs in recent untreated diabetes mellitus and the response of this condition to 1—2 weeks of carbohydrate restriction

It would be of some interest to know whether the hormonal and metabolic situation shown to exist here in obese and non-obese diabetics, alters with the further course of the disease. In the case of young patients this project is complicated by the fact that in general, insulin treatment must be instituted. In obese patients in whom continued dietary treatment is often sufficient, it should in many cases be possible to answer the above question. It would be of particular interest to know whether SILA and SF run a parallel course in patients whose diabetic anomaly of metabolism disappears when the body weight is reduced to normal (Newburgh diabetes) and likewise in patients in whom this cannot be achieved A fall in SF and/ or a rise in SILA in the blood could explain the favourable effect of loss of weight in the former group.

Summary

A study was performed on the glucose tolerance and the fasting concentrations of serum insulin-like activity (SILA) and

sulphation factor (SF) in diabetic patients before and after 1—2 weeks of carbohydrate restriction, leading to a fall in the 24-hour blood sugar level.

In recent untreated diabetes mellitus in obese patients, high SILA and tendency to high SF were found in the blood. Restricting carbohydrate ingestion caused a fall in the blood sugar level as well as a fall in SILA and SF.

In recent untreated diabetes mellitus in non-obese patients, the SILA values were found to be normal and the SF values were not high. Restricting carbohydrate ingestion caused the blood sugar level to fall here likewise, but SILA and SF remained unchanged.

Intravenous glucose tolerance was the same in both obese and non-obese diabetics, and remained unaffected on restricting carbohydrate ingestion.

These findings are compatible with the assumption that in obese patients, diabetes mellitus is based primarily on an overproduction of growth hormone, while in the non-obese it is a manifestation of hypoinsulinism.

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Gastric Acid Secretion Before and After Hypophysectomy in Man

By

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Animal experiments indicate that the majority of gastrointestinal glands do not preserve their structure and function after various disturbances of the endocrine system. When considering the pituitary hormones and the gastric mucosa, it has been shown that hypophysectomized rats have atrophy of the gastric glands, which cannot be explained by the diminished food intake (9) Morphological changes occur in the parietal as well as the chief cells and the volume of gastric juice and the amount of pepsin secreted are reduced (1) In man, diminished gastric secretion has been observed in hypophyseal insufficiency as well as in Addison's disease (11) Moreover treatment with pituitary adrenocorticotrophic hormone may increase gastric secretion (8)

The present report describes observations on the secretion of hydrochloric acid before and after hypophysectomy in man. The "maximal" acid secretion in response to a large dose of histamine was studied as it gives information about the number of active parietal cells (12)

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Material

Gastric acid secretion was studied in 8 cases, before and after hypophysectomy The patients examined were 4 women with mammary carcinoma and skeletal metastases, 4 patients with diabetes mellitus (2 males and 2 females) with proliferative retinopathy and impending total blindness. A random selection of cases was made from larger material referred to the hospital for eventual hypophysectomy A complete examination of the endocrine system was carried out before and after operation, in order to evaluate whether the removal of the anterior lobe was complete or not. Hypophysectomy was performed by the trans-antro-sphenoidal approach (10)

After operation all 8 cases were given 25 mg cortisone or cortisol acetate daily by mouth. Five cases were judged to have a complete loss of anterior lobe function with signs of thyroid and gonadal insufficiency in addition to that of the adrenal cortex. Four of the 5 "complete" cases were examined after cortisone substitution only Three of these 4 cases and the 5th complete case were also studied during substitution with 0.1—0.2 mg 1-thyroxine in addition to cortisone. The three incomplete cases

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Table II. Gastric secretion of acid in three patients before and after incomplete hypophysectomy

Case	Age (yrs)	Sex	Diagnosis	Gastric secretion of acid after maximal histamine stimulation		
				Before hypophysectomy Acid (mEq/h)	After incomplete hypophysectomy Subst. with cortisone	
					Time after op. (months)	Acid (mEq/h)
E.G.	40	♀	Acromegaly + mammary carcinoma	13.2	2	22.2
B.C.	33	♂	Diabetes mellitus with retinopathy	0.08	7	3.7
M.P.	32	♀	Diabetes mellitus with retinopathy	0	15	14.2

change in the other two. In the three incompletely hypophysectomized patients and in one patient kept on cortisone and thyroxine from the day of operation the gastric secretion of HCl was actually higher after operation. Especially striking was the increase in secretion in two diabetics, who before operation had low or absent HCl secretion (6).

It should be mentioned that the maximal histamine secretion test appears to have a good reproducibility, the difference between results of duplicate determinations not exceeding 12 % (6). The changes observed after hypophysectomy are therefore considered to be significant.

Discussion

The present investigation shows that the maximal HCl secretion is influenced by hypophysectomy. The HCl output after maximal histamine stimulation is regarded as a measure of the functioning parietal cell mass (5). It seems therefore likely that the 50 % reduction of HCl output after complete removal of the pituitary gland is the result of a functional or anatomical loss of parietal cells. This would be analogous to the circumstances in various animal species (2, 4, 13). Friedman (7) recorded regressive changes in the parietal cells and a reduction by 50 % of their total mass in hypophysectomized rats.

Four completely hypophysectomized patients were examined after adequate substitution with cortisone and thyroxine. In spite of this the maximal secretion of HCl was lower than before operation in 3 cases, while in the 4th case, a patient with diabetes the secretion was higher after operation than before. In two incompletely operated patients with diabetes there was also a distinct increase in the maximal acid output. The divergent behaviour of these three cases may depend on the diabetic illness as several factors may influence gastric function in this disease (5).

Animal experiments on the effects of hormonal substitution therapy on gastric function after hypophysectomy have given inconclusive results. Cortisone, thyr

Table I. Gastric secretion of acid in five patients before and after complete hypophysectomy

Case	Age (yrs)	Sex	Diagnosis	Gastric secretion of acid after maximal histamine stimulation				
				Before hypophysectomy Acid (mEq/h)	After hypophysectomy			
					Subst. with cortisone		Subst. with cortisone and thyroxine	
					Time after op. (months)	Acid (mEq/h)	Time after op. (months)	Acid (mEq/h)
I. S.	49	♀	Metastatic mammary carcinoma	15.3	2	6.3	—	—
E. J.	41	♀	Metastatic mammary carcinoma	11.0	10	6.3	20	6.4
E. O.	59	♀	Metastatic mammary carcinoma	14.3		4.9	15	8.6
L. W.	32	♀	Diabetes mellitus with retinopathy	6.6	—	—	7	10.4
W. R.	31	♂	Diabetes mellitus with retinopathy	15.5	4	9.4	44	9.[illegible]

[1] Normal values for: men 23.3 ± 1.4 mEq/h, women: 17.7 ± 1.6 mEq/h (5)

had normal thyroid function and received only cortisone substitution.

The first postoperative study of gastric secretion was performed 2—15 months after operation. In 3 cases a second test was made 15—44 months after operation.

Methods

The fasting subjects were examined in the morning. The patients with diabetes mellitus did not receive their morning dose of insulin on the day of the investigation. In hypophysectomized patients the daily maintenance dose of cortisone or hydrocortisone was given by intramuscular injection about 2 hours before the test.

The methods used were described by Dotevall (5). Briefly, the acid secretion in response to a subcutaneous dose of 0.04 mg/kg of histamine acid phosphate was measured. Untoward effects of histamine were counteracted by 50 mg of mepyramine maleate given intramuscularly 30 min. earlier. The output of acid was followed for 1 hour after histamine was given and expressed as milliequivalents of "total" acidity secreted during four 15 min. collection periods. "Free" HCl and "total" acidity was measured by electrometrical titration with 0.1 N NaOH to pH 3.5 and 8.0.

Circulatory effects of histamine (tachycardia and hypotension) were slight or absent in all subjects both before and after hypophysectomy. After the operation 2 patients reacted with a brief period of bradycardia and hypotension after histamine but this appeared to be a vaso-vagal reaction.

Results

The results are shown in tables I and II. After complete hypophysectomy the maximal secretion of HCl was reduced to an average of about 50 % in the 4 cases receiving cortisone substitution only. On repeated examination after addition of thyroxine substitution a slight rise in HCl secretion was seen in one case but no

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Polycythemia vera Following Treatment of Megaloblastic Anemia with Folic Acid

By

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Polycythemia vera following treatment of megaloblastic anemia is rare and has been reported in only a few cases (2 5, 6 7 8, 11 12, 13 14 16, 1 18, 19)

In all reports the cause of megaloblastic anemia was considered to be an Addisonian pernicious anemia. The patients were achlorhydric, responsive to treatment with B_{12} or liver extract, and showed no signs of any of the other conditions which are able to cause a megaloblastic anemia. Such evidence does not verify the diagnosis of Addisonian pernicious anemia beyond all doubt, but it is strongly suggestive.

Megaloblastic anemia during treatment with anticonvulsive drugs is not common, and in the past only about 60 cases have been reported (4 9) In 1954 Badenoch (1) reported a hitherto undescribed form of megaloblastic anemia in two patients who were receiving diphenylhydantoin. This association was subsequently confirmed by others. In addition the use of related drugs has been implicated in the production of megaloblastosis, one such drug being Mysoline®. An excellent hematological response to large doses of folic acid occurred in most cases. Although the mechanism of the anemia in question remains obscure, it is suggested that the anticonvulsants may interfere with the utilization of folic acid even if the absorption of folic acid from the intestine is normal. However this group of patients seems suitable for assessing the value of FIGLU excretion following histidine loading as an index of folic acid utilization (10)

The present report concerns a patient who developed polycythemia following folic acid therapy for megaloblastic anemia caused by an anticonvulsive drug (Phenantoin)

Case report

A 55-year-old woman was admitted to medical department F Frederiksborg County Hospital, November 1961 with chief complaints of severe fatigue and slow cerebration. Her previous history was as follows

There was no familiar disposition to blood disorders.

From 1938 she developed symptoms of epilepsy and she suffered equally from attacks of absences and grand mal.

Her epilepsy untreated for several years, was later considered as genuine type. From 1943 she was treated with Phenemal, Phenan-

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oxine, thyroid extract and growth hormone when given alone or in various combinations, may partially prevent the gastric mucosal atrophy in rats (2). Full return to normal may be obtained after transplantation of the pituitary gland.

The present material is small but the results provide evidence that the pituitary gland influences gastric function in man as in other species. The different behaviour of gastric secretion in patients with diabetes after hypophysectomy should be studied further.

Summary

The gastric acid secretion after maximal histamine stimulation was studied in 4 cases of metastasizing mammary carcinoma and 4 cases of diabetes mellitus with retinopathy. After complete hypophysectomy and substitution with cortisone the HCl secretion fell to about 50 % of the preoperative value. A reduced secretory response was also seen in 3 or 4 patients given cortisone and thyroxine as substitution after a complete operation. In 3 cases the hypophysectomy was judged to be incomplete; in these cases the secretory response increased following hypophysectomy, particularly so in two patients with diabetes, who had no or very low acid secretion before operation.

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and small intestine were normal. An achlorhydria had now developed. Unfortunately Schilling's tests were not performed on account of the patient's incooperability.

Up to now the patient has been treated with Phenantoin for 14 years. So a disturbance in her folic acid utilization could be expected. However the folic acid concentration in serum was normal (0.23 γ/ml) (normal values > 0.018γ/ml).

A disturbed folic acid metabolism was now established by examination of formiminoglutamic acid (FIGLU) excretion in the urine after administration of 15 g Histidine, very elevated value (2,700 μmol) being found (normal values < μ160 mol)[1].

The patient was now treated with folic acid 10 mg × 3 and iron perorally. Unfortunately the antiepileptic treatment was altered from Phenantoin to Mysoline® simultaneously with the beginning of folic acid administration. However disturbances in the folic acid utilization may be caused by both drugs.

During folic acid therapy her blood state was normalized and the FIGLU content in the urine fell to normal values (70 μmol).

On May 1962 the patient was admitted to the hospital again for controlling procedures. Surprisingly polycythemia vera had developed. Laboratory findings showed hemoglobin concentration 18.5 g/100 ml, red blood cell count 6.89 mill./mm³, white blood-cell count 16,000/mm³, platelet count 280,000/mm³, vitamin B_{12} in serum 300 μμg/ml, ESR 1 mm/h. Furthermore, radioactive sodium chromate blood-volume studies demonstrated an increase above normal in blood volume and red cell mass (6,900 ml and 3,350 ml respectively). Physical examination gave no further information, and in particular liver and spleen were not palpable. After reduction of her folic acid dose to 5 mg × 3 for some months, the hemoglobin concentration fell to normal values, as did red blood cell, white blood cell and platelet counts.

She was last seen in the hospital September 1962 where she continued to do well during treatment with folic acid 5 mg × 3 and unchanged anticonvulsive therapy.

Details of blood states during all the history are given in fig. 1.

We are grateful to dr. Kjell Jacobsson, M. D. Centrallasarettet, Växjö, Sweden.

Discussion

The present case is interesting considered from two points of view. In spite of a normal concentration in serum, the folic acid utilization was insufficient because of therapy with Phenantoin or Mysoline® or both. During treatment with folic acid and Mysoline® the excretion of FIGLU in the urine diminished and finally reached normal values. Two months later a polycythemia vera occurred. The development of polycythemia vera in this patient was established not only by the presence of the elevated red blood-cell, white blood-cell and platelet counts, but also by radioactive sodium chromate blood-volume studies, which demonstrated an increase above normal in the blood volume and red cell mass.

Current hypotheses on the occurrence of polycythemia during treatment of megaloblastic anemia are:

1) Erythremia may be caused by the antianemic drug.

2) The two diseases may tend to manifest themselves in such a sequence in the same patient without causal relationship.

3) The tendency to polycythemia may have been veiled by the anemic state. After restoration of the hematopoesis by treating the megaloblastic anemia the polycythemia becomes manifest.

4) An instability of the hematopoietic tissue in the patient may be suggestive.

The development of polycythemia after treatment of megaloblastic anemia with liver extract or vitamin B_1 has been noted only infrequently in the past. In some of the earlier case reports of polycythemia occurring after treatment of megaloblastic anemia with liver extract, no mention was made of leucocytoses and thrombocythemia commonly associated with polycythemia. In some of these patients the polycythemic red blood-cell

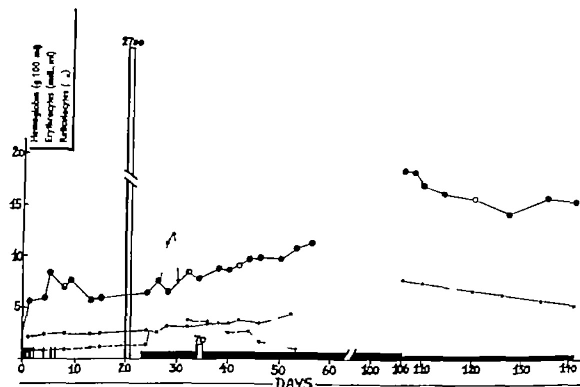


Fig 1 Details of blood states.

| Blood transfusion
○—○ Hemoglobin (g/100 ml)
●—● Erythrocytes (mill/ml)
●—● Reticulocytes (%)
□ FIGLU in urine (μmol)
■ Folic acid treatment.

toin was not given before 1947 The first information of her blood state was dated 1945 where the hemoglobin concentrations were 11.5 g/100 ml and 13 g/100 ml respectively

In 1949 she was referred to the medical department, Frederiksborg County Hospital, because of ecchymoses. However there were no obvious signs of blood disease (ESR 12 mm/h, red blood-cell count and platelet count normal (4 18 mill./mm³ and 205,500/mm³ respectively))

In 1955 she was referred on the same department because of a severe anemia. Her hemoglobin concentration was 4.5 g/100 ml, red blood-cell count 2.52 mill./mm³ white blood-cell count 4 960/mm³ serum iron 0.243 mg %

Bone marrow obtained by sternal puncture, however showed signs of a pernicious anemia, but no achlorhydria was found.

The patient was now treated with vitamin B_{12}, intrinsic factor and iron without effect. Only treatment with liver extract (Hepsol fortior®) gave a rise, albeit insufficient, in hemoglobin concentration and reticulocytes. Her antiepileptic treatment with Phenemal and Phenantoin continued unaltered. After leaving the hospital her blood state was controlled elsewhere.

On admission in 1961 physical examination revealed a deeply anemic and slow cerebrated patient somewhat overweight. Her tongue was normal, her spleen and liver could not be palpated. Neurologic examination including vibration sense was normal.

Laboratory findings

On admission hemoglobin concentration was 4 5 g/100 ml, red blood-cell count white blood-cell count and platelet count 2 mill./mm³ 4 440/mm³ and 42,300/mm³ respectively Serum iron elevated as earlier (0.270 mg %) vitamin B_{12} in serum 285 μμg/ml. Her colour index was not elevated but a new bone marrow specimen gave evidence of a pernicious anemia as earlier X-ray photos of ventricle

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values reverted to normal when the dosage of liver extract was reduced (3)

As far as we know polycythemia following treatment of megaloblastic anemia with folic acid has never been reported

In most cases in the literature the latent tendency for polycythemia vera may have been evoked by the anemic state or alternatively the two diseases tended to manifest themselves independently in such a sequence.

Most investigators therefore agree that the occurrence of both diseases in the same patient is caused by chance.

In our patient, however no evidence of a polycythemic state was found before the treatments with anticonvulsive drugs, vitamin B_{12} and folic acid The evolution of one myeloproliferative disorder into another is not unusual. Thus, polycythemia vera myeloid metaplasia and myelotic leukemia may follow or imperceptibly merge into one another Pernicious anemia may also be followed by myelotic leukemia, and the incidence of myelotic leukemia in the relatives of patients with pernicious anemia has been reported to be higher than in the general population Therefore an instability of the hematopoietic tissue in some patients is conceivable (15)

In our patient, however the hemoglobin concentration fell to normal values after reduction of the folic acid dose. At present no common pathophysiological basis is evident for the occurrence of polycythemia vera and megaloblastic anemia in the same patient.

Summary

A case of megaloblastic anemia caused by anticonvulsive drug treatment and developing polycythemia vera during treatment with folic acid is reported.

[...] of folic acid utilization was demonstrated by FIGLU tests, which showed falling values during administration of folic acid. Besides of red blood-cell, white blood-cell and platelet counts the polycythemia was verified by radioactive sodium chromate blood volume studies.

No evidence of polycythemia before the treatments with anticonvulsive drugs, vitamin B_{12} and folic acid was found.

The mechanism of the different disorders is briefly discussed.

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